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# WILLIAMS HEMATOLOGY

# Malignant Lymphoid Diseases

# Williams Hematology Malignant Lymphoid Diseases

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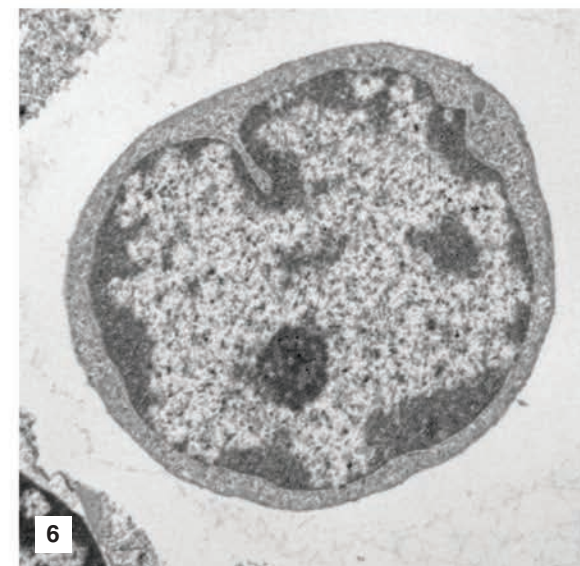
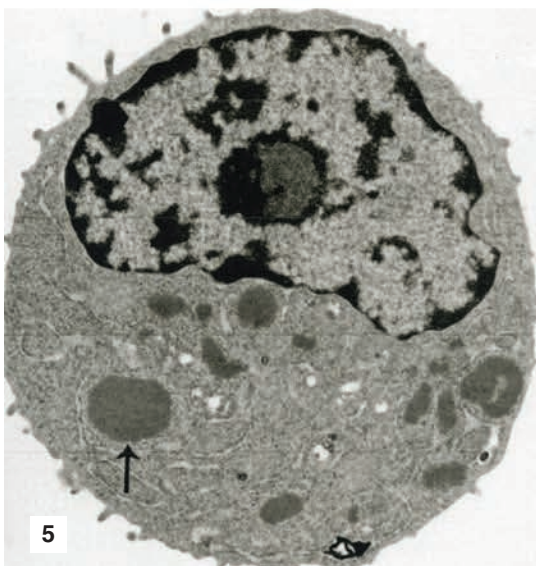
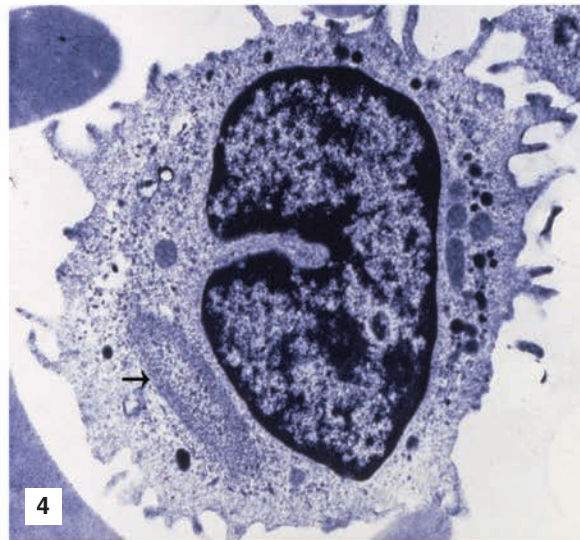
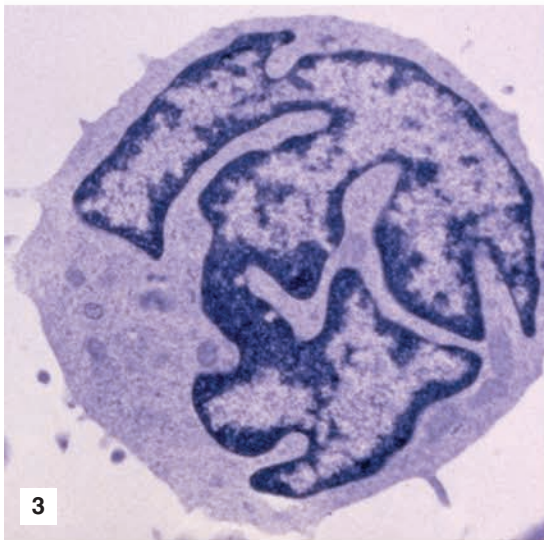
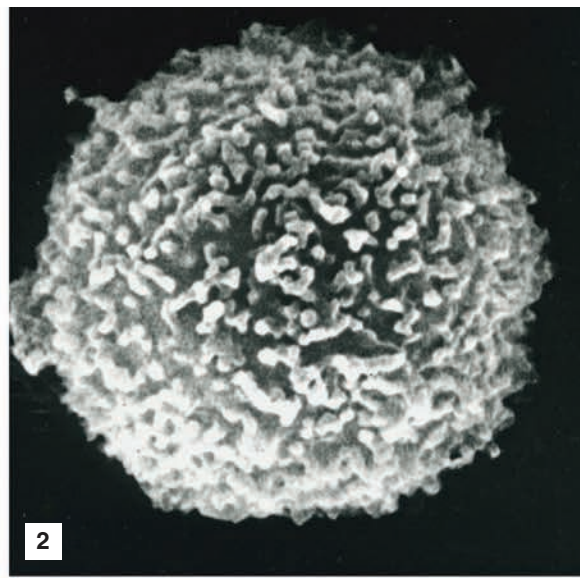
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**William J. Williams, MD**  
**1926 – 2016**

Medical educator, investigator, physician, mentor, academic leader,  
colleague, and the founding editor of *Williams Hematology*





1. Transmission electron micrograph (TEM) of a normal blood lymphocyte. 2. Scanning electron micrograph (SEM) of a normal blood lymphocyte. 3. TEM of Sézary cell in a patient with the erythrodermic type of cutaneous T-cell lymphoma. Note the cell's characteristic profoundly misshaped (cerebriform) nucleus. 4. TEM of a hairy cell. Arrow indicates a ribosome-lamella complex. This structure is not specific for hairy cell leukemia but is found in a variable proportion of hairy cells in about 50 percent of cases examined by TEM. Frequent cytoplasmic membrane, "hairy," projections. 5. TEM of plasmablast (undifferentiated myeloma cell). Arrow points to a Russell body. 6. A lymphoblast from the marrow of a patient with acute lymphoblastic leukemia. Very high nuclear-to-cytoplasmic ratio. Prominent nucleolus. The nucleus is virtually all euchromatin (likely transcriptionally active). (Reproduced with permission from Lichtman's Atlas of Hematology, [www.accessmedicine.com](http://www.accessmedicine.com).)

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# PREFACE

Bifurcation is an essential feature of biology. It underlies differentiation as one cell, through a process of mitosis accompanied by altered gene expression, forms two distinct cell lineages. The hematopoietic system is a dramatic example of this phenomenon. A single lymphohematopoietic stem cell, can over the course of several bifurcations, differentiate and then mature into at least 11 unique functional cells. In some cases, these cells can mature further into different phenotypes influenced by the environment in which they reside. Consider, for example, the monocytes, Kupffer cells, osteoclasts, microglia, and alveolar macrophages.

One of the critical points of hematopoietic bifurcation is the differentiation of the lymphohematopoietic stem cell into the common myeloid and common lymphoid progenitor. It is at this point that differentiation into these distinct lineages separates hematology into two specialized areas of research and clinical practice: the myeloid and lymphoid neoplasms. Unlike most of the maturing myeloid cells, the lymphoid cells do not lose their mitotic capability. This requirement for continued replication and repair of DNA, along with the rearrangements required of immunoglobulin and T-cell receptor genes during maturation, provides the risk of neoplastic gene mutations; these requirements result in a panoply of lymphocytic neoplasms, grossly divided into B-lymphocyte, T-lymphocyte, and natural killer cell tumors. The complexity of this array is extensive, with over 70 specific lymphocytic tumors in the 2016 World Health Organization classification of lymphocytic malignancies.

The lymphoid neoplasms are the subject of this text. Neoplasms originating in the lymphoid progenitor cell hierarchy constitute the lymphomas and lymphocytic leukemias. These tumors afflicted over 105,000 Americans and resulted in over 23,000 deaths in 2017. Their effects worldwide are dramatically larger. It is these compelling numbers that prompted the editors to prepare a “breakaway” text on the malignant lymphocytic neoplasms, based on the chapters that discussed these diseases in the ninth edition of *Williams Hematology*. Approximately 3 years have passed since those chapters were written. The editors asked the authors of these 21 chapters to revise and update them in the light of three recent developments: an expanded classification of the lymphocytic neoplasms by the World Health Organization, advances in the understanding of biology and genetics of these tumors, and advances in therapeutic approaches to the lymphomas and lymphocytic leukemias. The authors have graciously and expeditiously done so. With their help and expertise, we can now provide a timely text that covers the lymphomas and lymphocytic leukemias.

It is hoped the reader, from the accessibility of these new versions of the chapters, will derive benefit in their research, clinical practice, and learning.

Marshall A. Lichtman  
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# CHAPTER 1

## CLASSIFICATION OF MALIGNANT LYMPHOID DISORDERS

Robert A. Baiocchi

### SUMMARY

This chapter outlines the category of preneoplastic and neoplastic lymphocyte and plasma cell disorders. It introduces a framework for evaluating neoplastic lymphocyte and plasma cell disorders, outlines clinical syndromes associated with such disorders, and guides the reader to the chapters in the text that discuss each of these disorders in greater detail.

### ● CLASSIFICATION

Lymphocyte and plasma cell malignancies present a broad spectrum of different morphologic features and clinical syndromes (Table 1-1). Lymphocyte neoplasms can originate from cells that are at a stage prior to T- and B-lymphocyte differentiation from a primitive stem cell or from cells at stages of maturation after stem cell differentiation. For example, acute lymphoblastic leukemias arise from an early lymphoid progenitor cell that may give rise to cells with either B- or T-cell phenotypes (Chap. 2), whereas chronic lymphocytic leukemia arises from a more mature B-lymphocyte progenitor (Chap. 3) and myeloma from progenitors at even later stages of B-lymphocyte maturation (Chap. 18). Disorders of lymphoid progenitors may result in a broad spectrum of lymphocytic diseases, such as B- or T-cell lymphomas (Chaps. 9 and 15), hairy cell leukemia (Chap. 4), prolymphocytic leukemia (Chap. 3), natural

**Acronyms and Abbreviations:**  $\alpha/\beta$  TCR, T-cell-receptor genes encoding the  $\alpha$  and  $\beta$  chains of the T-cell receptor; *ALK*, gene encoding anaplastic lymphoma kinase; *BCL2*, gene encoding B-cell chronic lymphocytic leukemia (CLL)/lymphoma 2; *BCL6*, gene encoding B-cell chronic lymphocytic leukemia (CLL)/lymphoma 6; *clg*, cytoplasmic immunoglobulin; *EBER*, Epstein-Barr-virus-encoded RNA; *EBV*, Epstein-Barr virus;  $\gamma/\delta$  TCR, T-cell-receptor genes encoding the  $\gamma$  and  $\delta$  chains of the T-cell receptor; *HL*, Hodgkin lymphoma; *HLA*, human leukocyte antigen; *HTLV-1*, human T-cell leukemia virus type 1; *HHV8*, human herpes virus 8; *Ig*, immunoglobulin; *IgR*, immunoglobulin gene rearrangement; *IL*, interleukin; *MALT*, mucosa-associated lymphoid tissue; *MUM1*, gene encoding multiple myeloma oncogene 1; *neg.*, negative; *NK* cell, natural killer cell; *NOS*, not otherwise specified; *NPM*, gene encoding nucleophosmin; *PAX5*, paired box gene 5; *POEMS*, polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes; *REAL*, revised European-American lymphoma; *R-S*, Reed-Sternberg; *slg*, surface immunoglobulin; *slgD*, surface immunoglobulin D; *slgM*, surface immunoglobulin M; *TAL1*, gene encoding T-cell acute leukemia-1; *TCR*, T-cell receptor; *TdT*, terminal deoxynucleotidyl transferase; *Th2*, T-helper type 2; *WHO*, World Health Organization.

killer cell large granular lymphocytic leukemia (Chap. 5),<sup>1</sup> myeloma, and plasmacytoma (Chap. 18). Hodgkin lymphoma also is derived from a neoplastic B cell that has highly mutated immunoglobulin genes that are no longer expressed as protein (Chap. 8).

To provide a unified international basis for clinical and investigative work in this field, the International Lymphoma Study Group proposed a classification termed the *revised European-American Lymphoma* (REAL) classification (Chap. 6),<sup>2</sup> which was modified in 2001 and again in 2008 by the World Health Organization (WHO).<sup>3,4</sup> The REAL/WHO classification scheme makes use of the pathologic, immunophenotypic, genetic, and clinical features of given lymphocyte tumors to delineate them into separate disease entities (Table 1-1 and Chap. 7).<sup>5</sup> For some of these entities, the neoplastic lymphocytes have distinctive cytogenetic abnormalities, which can be identified using molecular techniques that are increasingly being used in clinical pathology laboratories.<sup>6,7</sup>

The REAL/WHO classification recognizes a basic distinction between nodular lymphocyte-predominant Hodgkin lymphoma and classic Hodgkin lymphoma, reflecting the differences in clinical presentation and behavior, morphology, phenotype, and molecular features (Chap. 8).<sup>3</sup> Studies have identified features that can be used to distinguish classical Hodgkin lymphoma from anaplastic large cell lymphoma and, to a lesser extent, between nodular lymphocyte-predominant Hodgkin lymphoma and T-cell/histiocyte-rich large B-cell lymphoma.

The updated WHO classification (summarized in Ref. 4) provided several revised guidelines for defining diseases such as chronic lymphocytic leukemia (CLL),<sup>8</sup> Waldenström macroglobulinemia,<sup>9</sup> plasma cell neoplasms,<sup>10</sup> and diffuse large B-cell lymphoma (DLBCL).<sup>11-14</sup> The classifications of several T-cell lymphomas were also refined, including enteropathy-associated T-cell lymphoma, anaplastic large cell lymphoma (*ALK* positive and *ALK* negative), and subcutaneous panniculitis-like T-cell lymphoma.<sup>4</sup> In 2014, a Clinical Advisory Committee meeting was held to review literature and provide an update prior to the preparation of the next WHO tumor monograph series. The update reviews major areas from the WHO 2018 edition that changed significantly<sup>14a</sup> and are summarized in Table 1-1.

### ● CLINICAL BEHAVIOR

Lymphomas of similar histology can have widely different spectra of associated clinical symptoms and clinical aggressiveness, making the categorization of lymphoid tumors impossible using a generic grading system based on morphology alone. For example, the neoplastic cells in mantle cell lymphoma appear smaller and more differentiated than those of anaplastic large cell lymphomas. However, the validation studies for the REAL classification revealed that patients with mantle cell lymphoma and anaplastic large cell lymphomas have 5-year survival rates of approximately 30 percent and approximately 80 percent, respectively.<sup>15,16</sup> Generally, T-cell lymphomas/leukemias have a more aggressive clinical behavior than B-cell lymphomas of comparable histology. The tendency for more aggressive disease also applies to lymphoid tumors derived from natural killer cells. A helpful distinction is to divide the lymphoid tumors into one of two categories, namely, indolent lymphomas versus aggressive lymphomas, based upon on the characteristics of the disease at the time of presentation and patients' life expectancy if the disease is left untreated.<sup>17,18</sup> Clinical studies have verified that the different disease categories defined in the REAL/WHO classification each can be segregated into one or the other of these two major categories (Tables 1-2 and 1-3, respectively).<sup>15</sup> Analyses of gene-expression patterns using microarray technology have enabled identification of subcategories within some of the disease categories defined by the REAL/WHO classification that have different tendencies for

**TABLE 1-1.** Classification of Lymphoma and Lymphoid Leukemia by the World Health Organization

| Neoplasm                                                                       | Morphology                                                               | Phenotype*                                                                                                            | Genotype†                                                                                                                                                                                                             |
|--------------------------------------------------------------------------------|--------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>B-CELL NEOPLASMS</b>                                                        |                                                                          |                                                                                                                       |                                                                                                                                                                                                                       |
| <b>Immature B-Cell Neoplasms</b>                                               |                                                                          |                                                                                                                       |                                                                                                                                                                                                                       |
| Lymphoblastic leukemia/lymphoma not otherwise specified (NOS) (Chap. 2)        | Medium-to-large cells with finely stippled chromatin and scant cytoplasm | TdT+, slg–, CD10+, CD13+/-, CD19+, CD20–, CD22+, CD24+, CD34+/-, CD33+/-, CD45+/-, CD79a+, PAX5+                      | Clonal DJ rearrangement of <i>IGH</i> gene T(17;19), <i>E2A-HLF</i> , <i>AML1</i> iAMP21 associated with poor prognosis                                                                                               |
| Lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities (Chap. 2) | See above                                                                | See above. B-ALL with t(9;22) with CD25 and more frequent myeloid antigens CD13, CD33                                 | See individual genetic features in B-ALL subtypes below                                                                                                                                                               |
| B-ALL with t(v;11q23); <i>MLL</i> rearranged                                   | See above                                                                | CD19+, CD10–, CD24–, CD15+                                                                                            | Multiple MLL (11q23) fusion partners, including <i>AF4</i> (4q21), <i>AF9</i> (9p22), and <i>ENL</i> (19p13). B-ALL with MLL translocations overexpress <i>FLT-3</i> . Poor prognosis                                 |
| B-ALL with t(12;21) (p13;q22); <i>TEL-AML1</i> ( <i>ETV6-RUNX1</i> )           | See above                                                                | CD19+, CD10+, CD34+. Characteristically negative for CD9, CD20, and CD66c                                             | t(12;21)(p13;q22) <i>ETV6-RUNX</i> translocation                                                                                                                                                                      |
| B-ALL with hyperdiploidy                                                       | See above                                                                | CD19+, CD10+, CD45–, CD34+                                                                                            | Numerical increase in chromosomes without structural abnormalities. Most frequent chromosomes +21, X, 14, and 4. +1, 2, 3 rarely seen. Favorable prognosis                                                            |
| B-ALL with hypodiploidy                                                        | See above                                                                | See above                                                                                                             | Loss of at least one or more chromosomes (range from 45 chromosomes to near haploid). Rare chromosome abnormalities. Poor prognosis                                                                                   |
| B-ALL with t(5;14) (q31;q32); <i>IL3-IGH</i>                                   | See above with increase in reactive eosinophilia                         | See above. Even rare blasts with B-ALL immunophenotype with eosinophilia strongly suggestive of this subtype of B-ALL | t(5;14)(q31;q32); <i>IL3-IGH</i> leading to overexpression of IL3. Unclear prognosis                                                                                                                                  |
| B-ALL with t(1;19) (q23;p13.3); <i>E2A-PBX1</i>                                | See above                                                                | CD10+, CD19+, cytoplasmic $\mu$ heavy chain. CD9+, CD34–                                                              | t(1;19)(q23;p13.3); leads to overexpression of <i>E2A-PBX1</i> fusion gene product interfering with normal transcription factor activity of E2A and PBX1                                                              |
| <b>Mature B-Cell Neoplasms</b>                                                 |                                                                          |                                                                                                                       |                                                                                                                                                                                                                       |
| <b>Leukemias</b>                                                               |                                                                          |                                                                                                                       |                                                                                                                                                                                                                       |
| Chronic lymphocytic leukemia/small lymphocytic lymphoma (Chap. 3)              | Small cells with round, dense nuclei                                     | slg+(dim), CD5+, CD10–, CD19+, CD20+(dim), CD22+(dim), CD23+, CD38+/-, CD45+, FMC-7–                                  | IgR+, trisomy 12 (~30%), del at 13q14 (~50%), 11q22–23, 17p13, and <i>IGHV</i> mutated status associated with poor prognosis. Mutations in <i>TP53</i> , <i>NOTCH1</i> , <i>SF3B1</i> , <i>ATM</i> , and <i>BIRC3</i> |
| Prolymphocytic leukemia (Chap. 3)                                              | ≥55% prolymphocytes                                                      | slg+(bright), CD5+/-, CD10–, CD19+, CD22+, CD23+/-, CD45+, CD79a+, FMC7+                                              | del13q.14(~30%); del17p (50%), IgR+                                                                                                                                                                                   |
| Hairy cell leukemia (Chap. 4)                                                  | Small cells with cytoplasmic projections                                 | slg+(bright), CD5–, CD10–, CD11c+(bright), CD19+, CD20+, CD25+, CD45+, CD103+, Annexin A+                             | <i>BRAF</i> mutations (~100%), IgR+ <i>MAP2K</i> mutations in <i>BRAF</i> wt                                                                                                                                          |
| <b>Lymphomas</b>                                                               |                                                                          |                                                                                                                       |                                                                                                                                                                                                                       |
| Lymphoplasmacytic lymphoma (Chap. 20)                                          | Small cells with plasmacytoid differentiation                            | clg+, CD5–, CD10–, CD19+, CD20+/-<br>Plasma cell population: CD38+, CD138+, clgM+                                     | IgR, 6q- in 50% of marrow-based cases [the t(9;14) was proved to be wrong], +4 (20%)                                                                                                                                  |

(Continued)

**TABLE 1-1.** Classification of Lymphoma and Lymphoid Leukemia by the World Health Organization (Continued)

| Neoplasm                                                                                      | Morphology                                                                                                                                                                                           | Phenotype*                                                                                                       | Genotype†                                                                                                                                                                                                                                                                              |
|-----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Mantle cell lymphoma: unmutated IgHV and SOX11+ (Chap. 11)                                    | Small-to-medium cells                                                                                                                                                                                | slgM+, slgD+, CD5+, CD10–, CD19+, CD20+, CD23–, cyclin D1+, FMC-7+, SOX11+                                       | Unmutated IgHV, SOX11+ MCL type. IgR, t(11;14)(q13;q32) (~100% by FISH), involving <i>CCND1</i> and IgH. Highly proliferative variants often show TP53 mutation, deletion of <i>INK4a/ART</i> and <i>p18INK4c</i> . <i>CCND1</i> – MCL show <i>CCND2</i> rearrangement in 50% of cases |
| Mantle cell lymphoma: mutated IgHV and SOX11–                                                 | See above                                                                                                                                                                                            | See above, SOX11–                                                                                                | See above                                                                                                                                                                                                                                                                              |
| Follicular lymphoma (follicle center lymphoma; FL, Chap. 10)                                  | Small, medium, or large cells with cleaved nuclei                                                                                                                                                    | slg, CD5–, CD10+, CD19+, CD20+(bright), CD23–/+, CD38+, CD45+                                                    | IgR, t(14;18)(q32;q21) (~85%) involving <i>BCL2</i> and IgH. Mutated 3q27 (5–15%, <i>BCL6</i> )                                                                                                                                                                                        |
| Predominantly diffuse FL with 1p36 deletion                                                   | See above                                                                                                                                                                                            | See above                                                                                                        | 1p36 deletion. Lacks <i>BCL2</i> rearrangement                                                                                                                                                                                                                                         |
| Duodenal-type FL                                                                              | See above                                                                                                                                                                                            | See above                                                                                                        | See above                                                                                                                                                                                                                                                                              |
| Pediatric-type FL                                                                             | See above                                                                                                                                                                                            | See above                                                                                                        | See above                                                                                                                                                                                                                                                                              |
| Nodal marginal zone B-cell lymphoma (Chap. 12)                                                | Small or large monocytoid cells                                                                                                                                                                      | slgM+, slgD–, clg+ (~50%), CD5–, CD10–, CD11c+/-, CD19+, CD20+, CD23–, CD43+/-                                   | IgR, commonly with trisomies 3, 7, and 18                                                                                                                                                                                                                                              |
| Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) type (Chap. 12) | See above                                                                                                                                                                                            | See above                                                                                                        | t(11;18)(q21;q21) involving <i>API2</i> , <i>MLT1</i> , or t(1;14)(p22;q32) involving <i>BCL10</i>                                                                                                                                                                                     |
| Splenic B-cell marginal zone lymphoma                                                         | Small round lymphocytes replace reactive germinal centers and/or villous lymphocytes in blood                                                                                                        | slgM+, slgD–, CD5+/-, CD19+, CD20+, CD23–, CD103–                                                                | IgR, allelic loss of chromosome 7q31–32 (40%)                                                                                                                                                                                                                                          |
| Splenic B-Cell Lymphoma, Unclassifiable                                                       |                                                                                                                                                                                                      |                                                                                                                  |                                                                                                                                                                                                                                                                                        |
| Splenic diffuse red pulp small B-cell lymphoma                                                | Blood: villous lymphocytes similar to SMZL. Marrow: intrasinusoidal infiltration. Spleen: monomorphous small-to-medium lymphocytes with round nuclei, vesicular chromatin, occasional small nucleoli | CD20+, DBA.44+, IgG+/IgD–, CD25–, CD5–, CD103–, CD123–                                                           | T(9;14)(p13;q32) involving <i>PAX5</i> and <i>IGH</i>                                                                                                                                                                                                                                  |
| Hairy cell leukemia variant                                                                   | Hybrid features of prolymphocytic leukemia and classic hairy cell leukemia                                                                                                                           | slg+(bright), CD5–, CD10–, CD11c+(bright), CD19+, CD20+, CD25–, CD45+, CD103+, FMC7+, CD123–, annexin A1–, TRAP– | BRAF mutation negative                                                                                                                                                                                                                                                                 |
| Diffuse Large B-Cell Lymphoma (DLBCL; Chap. 9)                                                |                                                                                                                                                                                                      |                                                                                                                  |                                                                                                                                                                                                                                                                                        |
| DLBCL NOS                                                                                     |                                                                                                                                                                                                      |                                                                                                                  |                                                                                                                                                                                                                                                                                        |
| Common Morphologic Variants:                                                                  |                                                                                                                                                                                                      |                                                                                                                  |                                                                                                                                                                                                                                                                                        |
| Centroblastic                                                                                 | Medium-to-large lymphoid cells with vesicular nuclei containing fine chromatin. Multiple nucleoli                                                                                                    | slgM+, slgD+/-, CD5–, CD10–/+, CD19+, CD20+, CD22+, CD79a+, CD45+, PAX5+                                         | IgR, 3q27 abnormalities and/or t(3;14)(q27;q32) involving <i>BCL6</i> (~30%) or t(14;18)(q32;q21) (~25%) involving <i>BCL2</i>                                                                                                                                                         |
| Immunoblastic                                                                                 | >90% of cells are immunoblasts with central nucleolus                                                                                                                                                | See above. May express CD30+                                                                                     | See above                                                                                                                                                                                                                                                                              |

(Continued)



**TABLE 1-1.** Classification of Lymphoma and Lymphoid Leukemia by the World Health Organization (Continued)

| Neoplasm                                                     | Morphology                                                                                                                                                                    | Phenotype*                                                                                                                                                                                | Genotype†                                                                                                                                                              |
|--------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Anaplastic                                                   | Very large round, oval, or polygonal cells with bizarre pleomorphic nuclei resembling R-S cells                                                                               | See above. Often CD30+                                                                                                                                                                    | See above                                                                                                                                                              |
| Molecular Subgroups                                          |                                                                                                                                                                               |                                                                                                                                                                                           |                                                                                                                                                                        |
| Germinal center B-cell-like (GCB)                            | See above                                                                                                                                                                     | See above                                                                                                                                                                                 | See above; MYC and BCL2 co-expression considered new prognostic marker                                                                                                 |
| Activated B-cell-like (ABC)                                  | See above. Often with more immunoblastic morphology                                                                                                                           | See above                                                                                                                                                                                 | t(14;18) (35%), 12q12 (20%), IG mutation, BCL2 rearrangement (20–25%), Rel amplification (15%). Amplification of microRNA-17-92 cluster                                |
| Immunohistochemical subgroups                                |                                                                                                                                                                               |                                                                                                                                                                                           | Gain of 3q (26%), 9p (6%), 12q12 (5%), NF-κB activation                                                                                                                |
| CD5-positive DLBCL                                           | See above                                                                                                                                                                     | See above. CD5+                                                                                                                                                                           | t(11;14) and t(14;18) negative. +3 and gain on chromosome 16/16p and 18/18q common. Deletion p16/INK4a                                                                 |
| Nongerminal center B-cell-like (non-GCB)                     | See above                                                                                                                                                                     | See above                                                                                                                                                                                 | See above. Uniform FOXP1 expression with IRF4/MUM1 and BCL6 expression                                                                                                 |
| T-cell/histiocyte-rich large B-cell lymphoma                 | See above; large cells dispersed among nonepithelioid histiocytes. Background lymphocytes are predominantly T cells                                                           | See above; negative for CD15, CD30, CD138. Background has CD68+ histiocytes and CD3+/CD5+ T cells                                                                                         | See above                                                                                                                                                              |
| Primary DLBCL of the CNS                                     | See above                                                                                                                                                                     | See above; positive for CD20, CD22, CD79a. CD10 expression present in 10–20% of cases. See above; BCL6 expression in 60–80% and IRF4/MUM1 in 90% of cases                                 | High burden of somatic hypermutations. + Mutations in <i>BCL6</i> , <i>PIM1</i> , <i>MYC</i> , <i>Rho/TTFn</i> , and <i>PAX5</i>                                       |
| Primary cutaneous DLBCL, leg type                            | See above                                                                                                                                                                     | See above; high percentage of tumor cells express BCL2, BCL6, IRF4/MUM1, FOXP1                                                                                                            | See above; deletion of 9p21.3 containing <i>CDKN2a</i> , <i>CDKN2b</i> , and <i>MTAP</i> in >60% cases                                                                 |
| EBV+ DLBCL, NOS                                              | See above; R-S-like cells present                                                                                                                                             | See above; EBV+ with expression of LMP1, EBNA2 in 90% of cases. Malignant cells often CD30+ and CD15–                                                                                     | See above, uniformly EBV+                                                                                                                                              |
| Primary mediastinal (thymic) large B-cell lymphoma (Chap. 9) | Variable from case to case. Medium-to-large cells often with pleomorphic nuclei (R-S-like cells)                                                                              | slg–, CD5–, CD10–/+, CD15–, CD19+, CD20+, CD22+, CD23+, CD30+ (80%), CD45+, CD79a+, IRF4/MUM1 (75%). Variable BCL2 (50–80%) and BCL6 (45–100%) expression                                 | IgR+, gain of 9q24 (75%), gain 2p15 (50%)<br>Amplification of <i>REL</i> , <i>BCL11A</i> , <i>JAK2</i> , <i>PDL1</i> , <i>PDL2</i> . Transcriptome similar to CHL      |
| Intravascular large B-cell lymphoma                          | Neoplastic cells infiltrated within small-to-intermediate vessels of all organs                                                                                               | CD19+, CD20+, CD5 (38%), CD10 (13%). Lack of CD29 (β1 integrin) and CD54 (ICAM1) may account for intravascular growth pattern                                                             | IgR+, otherwise poorly characterized                                                                                                                                   |
| Large B-cell lymphoma with <i>IRF4</i> rearrangement         | See above                                                                                                                                                                     | See above                                                                                                                                                                                 | <i>IRF4</i> rearrangement                                                                                                                                              |
| ALK-positive large B-cell lymphoma                           | Sinusoidal growth pattern, monomorphic large immunoblast-like cells                                                                                                           | Strongly positive for ALK, CD138+, VS38+, cytoplasmic IgA or IgG                                                                                                                          | IgR+, t(2;17) <i>ALK/CLTC</i>                                                                                                                                          |
| Plasmablastic lymphoma                                       | Diffuse proliferation of immunoblasts with plasmacytic differentiation, frequent mitotic figures, monomorphic morphology common in HIV+ patients. Frequently extranodal, EBV+ | CD138+, CD38+, VS38C, IRF4/MUM1+, high Ki67, CD79a+ CD30+ in most cases. Negative for CD45, CD20, PAX5. Cytoplasmic Ig (50–70%). CD56 negative (if positive, suspect plasma cell myeloma) | IgR+, frequently Epstein-Barr virus-encoded RNA (EBER)+ (60–70%) but most cases negative for LMP1. HHV8+ status consistent with large B-cell lymphoma from MCD (below) |

(Continued)

**TABLE 1–1.** Classification of Lymphoma and Lymphoid Leukemia by the World Health Organization (Continued)

| Neoplasm                                                                                                | Morphology                                                                                                                                                                                                                                                                                        | Phenotype*                                                                                                                                                      | Genotype†                                                                                                                                         |
|---------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|
| Large B-cell lymphoma arising from multicentric, HHV8+ Castleman disease (MCD)                          | HHV8 MCD: B-cell follicles with involution and hyalinization of germinal centers with prominent mantle zones. Large plasmablastic cells within mantle zone<br>HHV8 plasmablastic lymphoma. Confluent sheets of HHV8+ LANA1+ cells effacing lymph node architecture. Extranodal involvement common | HHV8+, LANA1+, viral IL6+, cytoplasmic IgM, CD20+/- . Negative for CD79a, CD138, and EBV (EBER)                                                                 | Polyclonal IgM. IgHV unmutated. IL6R pathway activation. Cytogenetics poorly characterized                                                        |
| Primary effusion lymphoma                                                                               | Range of infiltrating cells with highly abnormal morphology, including immunoblastic, plasmablastic, anaplastic. Large nuclei with prominent nucleoli                                                                                                                                             | CD45+; lack expression of CD19, CD20, CD79a, slg                                                                                                                | IgR+ and hypermutated. No recurrent chromosomal anomalies                                                                                         |
| Burkitt lymphoma (Chap. 9)                                                                              | Medium cells arranged in diffuse, monotonous pattern. Basophilic cytoplasm, high proliferative index with frequent mitotic figures. “Starry sky” pattern present                                                                                                                                  | Positive for CD19, CD20, CD10, BCL6, CD38, CD77, and CD43. Negative for BCL2 and TdT. Ki67+ in nearly 100% of tumor cells                                       | t(8;14)(q24;q32), t(2;8)(q11;q24), or t(8;22)(q24;q11), involving Ig loci and C-MYC at 8q24. TCF3 or ID3 mutations in up to 70% of cases          |
| Burkitt lymphoma with 11q aberration                                                                    | See above                                                                                                                                                                                                                                                                                         | See above                                                                                                                                                       | See above; TCF3 or ID3 mutations                                                                                                                  |
| High-grade B-cell lymphoma NOS with MYC and BCL2 and/or BCL6 translocations                             | Medium, round cells with abundant cytoplasm. More variation in nuclear size and contour compared to BL. Commonly >90% Ki67+. Unlike BL, can show strong BCL2 expression                                                                                                                           | Same as above except slg-, clg+/-, and CD10-                                                                                                                    | Same as above except more typically expresses high levels of BCL2 and ~30% have BCL2 rearrangements (double-hit type)                             |
| B-cell lymphoma unclassifiable, features intermediate between DLBCL and classical Hodgkin lymphoma (HL) | Confluent, diffuse, sheet-like growth of pleomorphic cells within a fibrotic stroma. Pleomorphic cells resembling HL R-S-like cells and lacunar cells. Necrosis frequent                                                                                                                          | In contrast to HL, CD45+. Positive for CD30 and CD15                                                                                                            | Poorly characterized                                                                                                                              |
| <b>Plasma Cell Neoplasms</b>                                                                            |                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                 |                                                                                                                                                   |
| Monoclonal gammopathy of undetermined significance (MGUS)                                               | Marrow infiltrate with mature plasma cells comprising 1–9% of cellularity                                                                                                                                                                                                                         | M-protein <30 g/L, marrow <10% plasma cells, no end-organ damage. CD138+. Often difficult to demonstrate LC restriction because of small number of plasma cells | Abnormal cytogenetics rarely encountered in MGUS. FISH studies involving IgH occur in ~50% of cases: t(11;14), t(4;14). Del13q. Hyperdiploidy 40% |
| Plasma cell myeloma                                                                                     | Myeloma plasma cells seen in marrow arranged in interstitial clusters                                                                                                                                                                                                                             | slg+, CD5-, CD10-, CD19-, CD20-, CD38+(bright), CD45+/-, CD56+, CD117+(bright), CD138+(bright)                                                                  | IgR, commonly with complex karyotypes and/or t(6;14)(p25;q32) involving MUM1. t(11;14) seen in 15–25% of cases                                    |
| Extraosseous plasmacytoma                                                                               | Plasma cells in extraosseous organs must be distinguished from other lymphoproliferative disorders (i.e., MALT type)                                                                                                                                                                              | Same as plasma cell myeloma                                                                                                                                     | Same as above                                                                                                                                     |
| Solitary plasmacytoma of bone                                                                           | Plasma cells                                                                                                                                                                                                                                                                                      | Same as plasma cell myeloma                                                                                                                                     | Same as above                                                                                                                                     |

(Continued)



**TABLE 1-1.** Classification of Lymphoma and Lymphoid Leukemia by the World Health Organization (Continued)

| Neoplasm                                                                             | Morphology                                                                                                                                                                                                                                          | Phenotype*                                                                                                                         | Genotype†                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Monoclonal immunoglobulin deposition disease                                         | Prominent organ (kidney most common; occasionally liver, heart, nerve, blood vessels involved) deposits of nonamyloid, nonfibrillary, amorphous eosinophilic material that does not stain with Congo red. Heavy chain (HCDD) and light chain (LCDD) | LCDD is $\kappa$ light chain predominant. HCDD shows $\lambda$ chain predominance. Marrow may show abnormal $\kappa/\lambda$ ratio | HCDD with V $\lambda$ I overrepresentation. LCDD with V $\kappa$ IV variable region                                                                                                                                                        |
| <b>Hodgkin Lymphoma (HL)</b>                                                         |                                                                                                                                                                                                                                                     |                                                                                                                                    |                                                                                                                                                                                                                                            |
| Nodular lymphocyte predominant HL (Chap. 8)                                          | "Popcorn cells" with nuclei resembling those of centroblasts                                                                                                                                                                                        | BCL6+, CD19+, CD20+, CD22+, CD45+, CD79a+, CD15–, and rarely CD30+/-, Bob1+, Oct2+, PAX5+                                          | IgR, with high-level expression of BCL6                                                                                                                                                                                                    |
| <b>Classic HL (Chap. 8)</b>                                                          |                                                                                                                                                                                                                                                     |                                                                                                                                    |                                                                                                                                                                                                                                            |
| Nodular sclerosis HL                                                                 | R-S cells and lacunar cells dispersed in reactive lymphoid nodules                                                                                                                                                                                  | R-S cells typically are CD15+, CD20–/+, CD30+, CD45–, CD79a–, PAX5+(dim)                                                           | R-S cells generally express PAX5 and MUM1, variable expression of BCL6, and have IgR without functional Ig                                                                                                                                 |
| Lymphocyte-rich HL                                                                   | Few R-S cells with occasional "popcorn" appearance dispersed in lymphoid nodules                                                                                                                                                                    | Same as above                                                                                                                      | Same as above                                                                                                                                                                                                                              |
| Mixed-cellularity HL                                                                 | R-S cells dispersed among plasma cells, epithelioid histiocytes, eosinophils, and T cells                                                                                                                                                           | R-S cells typically are CD15+, CD20–/+, CD30+, CD45–, CD79a–                                                                       | R-S cells generally express PAX5 and MUM1, variable expression of BCL6, and have IgR without functional Ig                                                                                                                                 |
| Lymphocyte-depleted HL                                                               | Prominent numbers of R-S cells with effacement of the nodal structure                                                                                                                                                                               | Same as above                                                                                                                      | Same as above                                                                                                                                                                                                                              |
| <b>T-CELL NEOPLASMS</b>                                                              |                                                                                                                                                                                                                                                     |                                                                                                                                    |                                                                                                                                                                                                                                            |
| <b>Immature T-Cell Neoplasms</b>                                                     |                                                                                                                                                                                                                                                     |                                                                                                                                    |                                                                                                                                                                                                                                            |
| Lymphoblastic leukemia (Chap. 2)                                                     | Medium-to-large cells with finely stippled chromatin and scant cytoplasm                                                                                                                                                                            | TdT+, CD2+/-, cytoplasmic CD3+, CD1a+/-, CD5+/-, CD7+, CD10–/+, CD4+/CD8+ or CD4–/CD8–, CD34+/-                                    | Abnormalities in TCR loci at 14q11 (TCR- $\alpha$ ), 7q34 (TCR- $\beta$ ), or 7p15 (TCR- $\gamma$ ), and/or t(1;14)(p32-34; q11) involving TAL1                                                                                            |
| Lymphoblastic lymphoma (Chap. 2)                                                     | Same as above                                                                                                                                                                                                                                       | Same as above                                                                                                                      | Same as above                                                                                                                                                                                                                              |
| <b>Mature T- and NK-Cell Neoplasms</b>                                               |                                                                                                                                                                                                                                                     |                                                                                                                                    |                                                                                                                                                                                                                                            |
| <b>Leukemias</b>                                                                     |                                                                                                                                                                                                                                                     |                                                                                                                                    |                                                                                                                                                                                                                                            |
| T-cell prolymphocytic leukemia (Chap. 15)                                            | Small-to-medium cells with cytoplasmic protrusions or blebs                                                                                                                                                                                         | TdT–, CD2+, CD3+, CD5+, CD7+, CD4+ and CD8– is more common than CD4– and CD8+, but can be CD4+ and CD8+                            | $\alpha/\beta$ TCR rearrangement, inv14(q11;q32)(~75%). Inv14 in ~80% of cases. Translocations frequently involve TCL1A and TCL1B genes. +8q seen in ~75% of cases. del 11q23 and abnormalities with chromosome 6 (33%) and 17P (26%) seen |
| T-cell large granular lymphocytic leukemia (Chap. 5)                                 | Abundant cytoplasm and sparse azurophilic granules                                                                                                                                                                                                  | CD2+, CD3+, CD4 –/+, CD5+, CD7+, CD8+/-, CD16+/-, CD56–, CD57+/-                                                                   | $\alpha/\beta$ TCR rearrangement, $\gamma/\delta$ rearrangement can be seen. Subtypes with STAT3 and STAT5B mutations. STAT5B mutations associated with aggressive disease                                                                 |
| <b>Lymphomas/Lymphoproliferative Disorders</b>                                       |                                                                                                                                                                                                                                                     |                                                                                                                                    |                                                                                                                                                                                                                                            |
| Extranodal T/NK-cell lymphoma, nasal type ("angiocentric lymphoma"; Chaps. 5 and 15) | Angiocentric and angiodestructive growth                                                                                                                                                                                                            | CD2+, cytoplasmic CD3+, CD4–, CD5–/+, CD7+, CD8–, CD56+, EBV+                                                                      | TCR rearrangements usually neg., EBV present by <i>in situ</i> hybridization                                                                                                                                                               |

(Continued)

**TABLE 1-1.** Classification of Lymphoma and Lymphoid Leukemia by the World Health Organization (Continued)

| Neoplasm                                                                | Morphology                                                                                                               | Phenotype*                                                                                                                  | Genotype†                                                                                                                                                                             |
|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cutaneous T-cell lymphoma (mycosis fungoides; Chap. 14)                 | Small-to-large cells with cerebriform nuclei                                                                             | CD2+, CD3+, CD4+, CD5+, CD7+/-, CD8-, CD25-, CD26+                                                                          | $\alpha/\beta$ TCR rearrangements, complex karyotype common. STAT3 activation                                                                                                         |
| Sézary syndrome (Chap. 14)                                              | Same as above                                                                                                            | Same as above                                                                                                               | Same as above                                                                                                                                                                         |
| Angioimmunoblastic T-cell lymphoma <sup>34</sup>                        | Small-to-medium immunoblasts with clear-to-pale cytoplasm around follicles and high endothelial venules                  | CD3+/-, CD4+, CD10+, CXCL13+, PD-1+ (60–100%), EBV+                                                                         | $\alpha/\beta$ TCR rearrangement (75–90%), IgR (25–30%), trisomy 3 or 5 noted                                                                                                         |
| Peripheral T-cell lymphoma (not otherwise unspecified; Chap. 15)        | Highly variable                                                                                                          | CD2+, CD3+, CD5+, CD7-, CD4+/CD8- more often than CD4-/CD8+, which is more often than CD4+/CD8+                             | $\alpha/\beta$ TCR rearrangement                                                                                                                                                      |
| Subcutaneous panniculitis-like T-cell lymphoma <sup>35</sup>            | Variable size atypical cells with hyperchromasia infiltrating fat lobule                                                 | CD2+, CD3+, CD4-, CD5+, CD7-, CD8+, and cytotoxic molecules (perforin, granzyme B, and TIA1)                                | $\alpha/\beta$ TCR rearrangement                                                                                                                                                      |
| Enteropathy-associated T-cell lymphoma (EATL) type 1                    | Medium-to-large cells with prominent nucleoli, abundant pale cytoplasm invading mucosal membranes of the small intestine | CD3+, CD5-, CD7+, CD8+/-, CD4-, CD103+, TCR $\beta$ +/-, CD30+ (most cases)                                                 | <i>TRB</i> , <i>TRG</i> clonally rearranged. >90% <i>HLADQA1*0501</i> , <i>DQB1*0201</i> ; type 1: associated with celiac disease                                                     |
| Monomorphic epitheliotropic intestinal T-cell lymphoma                  | See above                                                                                                                | See above                                                                                                                   | Not associated with celiac disease                                                                                                                                                    |
| Indolent T-cell lymphoproliferative disorder of the GI tract            | See above; superficial monoclonal intestinal T-cell infiltrate                                                           | See above                                                                                                                   | See above                                                                                                                                                                             |
| Hepatosplenic T-cell lymphoma <sup>37-39</sup>                          | Small-to-medium cells with condensed chromatin and round nuclei                                                          | CD2+, CD3+, CD4-, CD5+, CD7+/-, CD8+/-                                                                                      | $\gamma/\delta$ TCR rearrangement, rarely $\alpha/\beta$ TCR rearrangement, isochromosome 7q                                                                                          |
| Adult T-cell leukemia/lymphoma (Chap. 2)                                | Highly pleomorphic with multilobe nuclei                                                                                 | CD2+, CD3+, CD5+, CD7-, CD25+, CD4+/CD8- more often than CD4-/CD8+                                                          | $\alpha/\beta$ TCR rearrangement, integrated HTLV-1                                                                                                                                   |
| Anaplastic large cell lymphoma ALK-positive                             | Large pleomorphic cells with "horseshoe"-shaped nuclei, prominent nucleoli, and abundant cytoplasm                       | TdT-, ALK1+, CD2+/-, CD3-/+ , CD4-/+ , CD5-/+ , CD7+/-, CD8-/+ , CD13-/+ , CD25+/-, CD30+, CD33-/+ , CD45+, HLA-DR+, TIA+/- | TCR rearrangement, t(2;5)(p23;q35) resulting in nucleophosmin— <i>anaplastic lymphoma kinase</i> fusion protein ( <i>NPM/ALK</i> ); other translocations involving 2p23 are also seen |
| Anaplastic large cell lymphoma ALK-negative                             | Similar morphologic spectrum to that seen in ALK+ ALCL. No small cell variant seen in ALK-                               | TdT-, ALK1-, CD2+/-, CD3-/+ , CD4-/+ , CD5-/+ , CD7+/-, CD8-/+ , CD13-/+ , CD25+/-, CD30+, CD33-/+ , CD45+, HLA-DR+, TIA+/- | TCR rearrangement, cytogenetic subsets with 6p25 rearrangements at <i>IRF4/DUSP22</i> locus                                                                                           |
| Primary cutaneous CD30+ anaplastic large cell lymphoma <sup>43,44</sup> | Anaplastic large cells as above in cutaneous nodules                                                                     | TdT-, CD2-/+ , CD3+/-, CD4+, CD5-/+ , CD7+/-, CD25+/-, CD30+, CD45+                                                         | TCR rearrangement but without t(2;5)(p23;q35)                                                                                                                                         |
| Breast implant-associated anaplastic large cell lymphoma                | Noninvasive disease associated with excellent outcome. Neoplastic cells confined to seroma fluid proximal to implant     | See above                                                                                                                   | See above                                                                                                                                                                             |
| Primary cutaneous acral CD8+ T-cell lymphoma                            | Indolent disease originating in the ear                                                                                  | See above; CD8+                                                                                                             | See above                                                                                                                                                                             |

(Continued)

**TABLE 1-1.** Classification of Lymphoma and Lymphoid Leukemia by the World Health Organization (Continued)

| Neoplasm                                                                             | Morphology                                                                                                                                                                                                                                                                                                    | Phenotype*                                                                                      | Genotype†                                                                    |
|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| Lymphomatoid papulosis                                                               | Three histologic subtypes (A, B, C). Type A: scattered clusters of large R-S-like cells admixed with histiocyte-rich infiltrate. Type B: rarely seen, epidermotropic infiltrate of small atypical cells with cerebriform nuclei (MF-like). Type C: monotonous large CD30+ T cells with few inflammatory cells | Type A and C have similar phenotype to C-ALCL. Type B phenotype CD3+, CD4+, CD8-, CD30-         | TCR rearrangement in 60% of cases. No t(2;5)(p23;135)                        |
| Primary Cutaneous Peripheral T-Cell Lymphomas, Rare Subtypes:                        |                                                                                                                                                                                                                                                                                                               |                                                                                                 |                                                                              |
| Primary cutaneous $\gamma/\delta$ T-cell lymphoma                                    | Epidermotropic, dermal, and subcutaneous histologic patterns. Neoplastic cells medium to large with coarse chromatin, frequent apoptosis/necrosis                                                                                                                                                             | CD3+, CD2+, CD5-, CD7+/-, CD56+. Most cases CD4-, CD8-                                          | TCRG, TCRD clonal rearrangement. EBV negative                                |
| Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma           | Variable histology ranging from lichenoid epidermotropism to deeper nodular infiltrates. Tumor cells small to medium with pleomorphic or blastic nuclei                                                                                                                                                       | CD3+, CD8+, granzyme B+, perforin+, TIA1+, CD45RA+/-, CD45RO-, CD2+/-, CD4-, CD5-, CD7+/-       | Clonal TCR rearrangement. EBV negative                                       |
| Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder              | Dense, diffuse, dermal infiltrates. Predominance of small/medium pleomorphic cells                                                                                                                                                                                                                            | CD3+, CD4+, CD8-, CD30-. No cytotoxic proteins expressed                                        | Clonal TCR rearrangement. EBV negative                                       |
| Systemic EBV+ T-cell lymphoma of childhood                                           | Infiltrating T cells are EBV+ but lack cytologic atypia. Erythrophagocytosis and histiocytosis seen frequently                                                                                                                                                                                                | CD2+, CD3+, CD56-, CD8+, EBER+                                                                  | Clonal TCR rearrangement. EBV+ with LMP1 expression                          |
| Hydroa vacciniforme-like lymphoproliferative disorder                                | Cutaneous presentation, small-to-medium cells without clear cytology atypical                                                                                                                                                                                                                                 | CD3+, CD8+, CD56+                                                                               | Clonal TCR rearrangement. EBV+ without LMP1                                  |
| <b>NATURAL KILLER (NK) CELL NEOPLASMS</b>                                            |                                                                                                                                                                                                                                                                                                               |                                                                                                 |                                                                              |
| Large granular lymphocytic leukemia (Chap. 5)                                        | Abundant cytoplasm and sparse azurophilic granules                                                                                                                                                                                                                                                            | TdT-, CD2+, CD3-, CD4-, CD5-/+ , CD7+, CD8-/+ , CD11b+, CD16+, CD56+, CD57+/-                   | No TCR rearrangement                                                         |
| Aggressive NK-cell leukemia <sup>1</sup>                                             | Same as above                                                                                                                                                                                                                                                                                                 | Same as above                                                                                   | No TCR rearrangement, EBV present                                            |
| Extranodal NK-cell lymphoma, nasal-type ("angiocentric lymphoma") <sup>1,45,46</sup> | Angiocentric and angiodestructive growth                                                                                                                                                                                                                                                                      | CD2+, cytoplasmic CD3 $\epsilon$ +, CD4-, CD5-/+ , CD7+, CD8-, CD56+                            | No TCR rearrangement, EBV present                                            |
| Immunodeficiency-Associated Lymphoproliferative Disorders                            |                                                                                                                                                                                                                                                                                                               |                                                                                                 |                                                                              |
| Lymphoproliferative disorders associated with primary immune disorders               | Range of morphology from reactive hyperplasia, polymorphous lymphoid infiltrate, to high-grade lymphomas. Lymphoma and HL morphology is similar to that seen in immune-competent patients                                                                                                                     | Immunophenotype similar to that seen in immune-competent patients with corresponding malignancy | FAS mutation seen in ALPS. Mutations in SAP/SLAM in XLP. ATM mutations in AT |

(Continued)

**TABLE 1–1.** Classification of Lymphoma and Lymphoid Leukemia by the World Health Organization (Continued)

| Neoplasm                                                                           | Morphology                                                                                                                                                                                                          | Phenotype*                                                                                                | Genotype†                                                                                                                      |
|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Lymphomas associated with HIV infection                                            | Similar to above. Typical histologic features seen in Burkitt lymphoma, HL, DLBCL. Lymphomas seen more frequently in HIV setting include primary effusion, plasmablastic, lymphomas, multicentric Castleman disease | Similar to above                                                                                          | <i>MYC</i> and <i>BCL2</i> translocations seen in DLBCL                                                                        |
| Posttransplant Lymphoproliferative Disorders (PTLDs)                               |                                                                                                                                                                                                                     |                                                                                                           |                                                                                                                                |
| Early lesions: plasmacytic hyperplasia (PH) and infectious mononucleosis (IM)–like | PH: numerous plasma cells, lymphocytes, and immunoblasts. IM: numerous immunoblasts on a background of T cells                                                                                                      | Similar to above                                                                                          | EBV+ in both IM and PH. Oligoclonal polyclonal IgH rearrangement. EBV+                                                         |
| Polymorphic PTLD                                                                   | Effacement of tissue architecture with infiltrate showing full range of B-cell maturation                                                                                                                           | Similar to above with exception that R-S cells in HLs often express CD30+, CD20+ but frequently are CD15– | Clonal <i>Ig</i> rearrangement. EBV+ by EBER ISH. Mutated IgH in 75% of cases                                                  |
| Monomorphic PTLD                                                                   | Similar to DLBCL, Burkitt lymphoma, or plasmacytoma morphology                                                                                                                                                      | Similar to above                                                                                          | EBV+/- . Clonal B cell or T cells. Cytogenetics frequently with <i>TP53</i> , <i>RAS</i> mutations, <i>BCL6</i> translocations |
| Other iatrogenic immunodeficiency-associated lymphoproliferative disorders         | Increased frequency of HL and lymphoproliferation with Hodgkin-like features. Histologic features can otherwise resemble the range of features seen in other immune deficiency–related LPDs                         | HL-like show CD20+, CD30+, CD15– or CD20–, CD30+, CD15+ staining. EBV is variably positive                | Same as above                                                                                                                  |

FISH, fluorescence *in situ* hybridization; IgR, immunoglobulin gene rearrangement; IgHV, immunoglobulin heavy chain variable region; MCD, multicentric Castleman disease; neg., negative; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NK, natural killer; R-S, Reed-Sternberg; SMZL, splenic marginal zone lymphoma; STAT, signal transducer and activator of transcription; TCR, T-cell receptor. Also see “Acronyms and Abbreviations” at the beginning of this chapter.

\*The immunophenotype revealed by immunohistochemistry and/or flow cytometry of surface antigens that typically are found for neoplastic cells of a given disorder are listed. If a CD antigen is indicated, then most of the neoplastic cells express that particular surface protein that is expressed by most tumor cells. CD antigens that have a minus (–) sign suffix are characteristically not expressed by the neoplastic cells of that disease entity. CD antigens that have a +/- sign suffix are not expressed by the neoplastic cells of all patients with that entity or are expressed at low or variable levels on the tumor cells. Antigens that have a -/+ sign suffix are expressed at very low levels or by the tumor cells of a minority of patients.

†The common genetic features associated with a given type of neoplasm are indicated. The numbers in parentheses provide the approximate proportion of cases that have the defined phenotype or genetic abnormality.

disease progression, survival, and/or response rates to standard therapies (Chap. 7).<sup>19–25</sup> An example of how gene-expression profiling has had a major impact on refining lymphoma diagnoses can be found with two newly defined working categories as “gray zone” lymphomas between Hodgkin lymphoma and primary mediastinal large B-cell lymphoma<sup>12,26</sup> and between Burkitt and DLBCL.<sup>13,14</sup> These new intermediate groups make clear distinctions between biologic and clinical features of conventional DLBCL and HL.

## ● ASSOCIATED CLINICAL SYNDROMES

### EARLY PRECURSOR LESIONS IN LYMPHOID NEOPLASMS

The 2008 WHO classification highlights several clinical, histologic, and immunophenotypic observations supporting the notion that lymphoid

neoplasms arise from clonal expansion and, ultimately, malignant transformation of precursor lesions. Monoclonal B-cell lymphocytosis (MBL) can be found in first-degree relatives of patients with CLL and in 5 to 15 percent of adults older than 60 years of age who present with lymphocytosis.<sup>27,28</sup> The documented rate of progression to CLL of 1 to 2 percent/year and immunophenotypic evidence of evolving CLL-like clones with cytogenetic anomalies suggest that mantle cell lymphoma may represent a potential precursor to CLL.<sup>29</sup> Other potential precursor lesions for follicular lymphoma and mantle cell lymphoma are currently under investigation.<sup>4</sup>

### ABNORMAL PRODUCTION OF IMMUNOGLOBULIN

When B lymphocytes undergo neoplastic transformation and clonal proliferation, they can secrete monoclonal proteins inappropriately

**TABLE 1-2. Indolent Lymphomas**

|                                                                                           |
|-------------------------------------------------------------------------------------------|
| Disseminated lymphomas/leukemias                                                          |
| Chronic lymphocytic leukemia                                                              |
| Hairy cell leukemia                                                                       |
| Lymphoplasmacytic lymphoma                                                                |
| Splenic marginal zone B-cell lymphoma (with or without villous lymphocytes)               |
| Plasma cell myeloma/plasmacytoma                                                          |
| Nodal lymphomas                                                                           |
| Follicular lymphoma                                                                       |
| Nodal marginal zone B-cell lymphoma (with or without monocytoid B cells)                  |
| Small lymphocytic lymphoma                                                                |
| Extranodal lymphomas                                                                      |
| Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) type |

(Chaps. 16 and 17). If the monoclonal protein is immunoglobulin (Ig) M, IgA, or a member of certain subclasses of IgG (e.g., IgG<sub>3</sub>), its presence may increase the viscosity of the blood, impairing blood flow through the microcirculation (Chaps. 18 and 20). This process may be impeded further by the associated homotypic erythrocyte aggregation (pathologic rouleaux) that often occurs in blood with a high concentration of immunoglobulin protein. Collectively, this situation may result in

**TABLE 1-3. Aggressive Lymphomas**

|                                                                                              |
|----------------------------------------------------------------------------------------------|
| Immature B-cell neoplasms                                                                    |
| B-cell lymphoblastic leukemia/lymphoma                                                       |
| Mature B-cell neoplasms                                                                      |
| Burkitt lymphoma/Burkitt cell leukemia                                                       |
| Diffuse large B-cell lymphoma                                                                |
| Follicular lymphoma grade III                                                                |
| Mantle cell lymphoma                                                                         |
| Immature T-cell neoplasms                                                                    |
| T-cell lymphoblastic lymphoma/leukemia                                                       |
| Peripheral T- and natural killer (NK) cell neoplasms                                         |
| T-cell prolymphocytic leukemia/lymphoma                                                      |
| Aggressive NK-cell leukemia/lymphoma                                                         |
| Adult T-cell lymphoma/leukemia (associated with HTLV-1 [human T-cell leukemia virus type 1]) |
| Extranodal NK/T-cell lymphoma                                                                |
| Enteropathy-associated T-cell lymphoma                                                       |
| Hepatosplenic T-cell lymphoma                                                                |
| Subcutaneous panniculitis-like T-cell lymphoma                                               |
| Peripheral T-cell lymphomas, not otherwise specified                                         |
| Angioimmunoblastic T-cell lymphoma                                                           |
| Anaplastic large cell lymphoma, primary, systemic                                            |
| Immune deficiency-associated lymphoproliferative disorders                                   |

the hyperviscosity syndrome, manifested clinically by headache, dizziness, diplopia, stupor, retinal venous engorgement, or frank coma (Chap. 20).<sup>30,31</sup>

Monoclonal immunoglobulin proteins also can interact with cell surfaces and impair granulocyte or platelet function or they can interact with coagulation proteins to impair their function in hemostasis. Excessive excretion of immunoglobulin light chains can lead to several types of renal tubular dysfunction and renal insufficiency (Chaps. 17 and 18). IgM deposited in glomerular tufts also can lead to renal disease (Chap. 20). Cryoglobulins (immunoglobulins that precipitate at temperatures below 37°C) can result in Raynaud syndrome, skin ulcerations, purpura, digital infarction, and gangrene. These manifestations result from immune complex formation, complement activation, and precipitation of cryoglobulins in cutaneous blood vessels. Excessive production of monoclonal immunoglobulin or immunoglobulin fragments in myeloma (Chap. 18) or in heavy-chain disease (Chap. 21) may lead to formation of amyloid, resulting in primary amyloidosis (Chap. 19).

Production of autoreactive antibodies spontaneously or in relationship to a B-lymphocyte neoplasm may lead to autoimmune hemolytic anemia, autoimmune thrombocytopenia, or, rarely, autoimmune neutropenia. Autoantibodies directed against tissues are implicated in the pathogenesis of diseases such as autoimmune thyroiditis, adrenalitis, encephalitis, and conditions with other organ involvement. Peripheral neuropathies as a result of demyelination can occur in patients with monoclonal immunoglobulin (Chaps. 17, 18, and 19). The neural injury often is related to antibody activity against myelin-associated glycoproteins or absorption by nerve tissue.<sup>31</sup> Rarely, the polyneuropathy is part of the polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes (POEMS) syndrome (Chap. 18).<sup>32</sup>

## MARROW AND OTHER TISSUE INFILTRATION

Well-differentiated malignant B lymphocytes, such as those found in the early stages of CLL or Waldenström macroglobulinemia, may infiltrate the marrow extensively, causing impairment of hemopoiesis. Eventually, however, massive infiltration of marrow by malignant B lymphocytes can suppress normal hemopoiesis, resulting in varying combinations of anemia, neutropenia, and/or thrombocytopenia (Chap. 3). Malignant B-lymphocyte proliferation or infiltration may result in any combination of splenomegaly and lymphadenopathy of either superficial or deep lymph nodes. DLBCLs tend to involve isolated lymph node groups (Chaps. 8 and 9), whereas low-grade lymphomas (follicular lymphoma) and lymphoproliferative disorders (CLL) tend to present with more diffuse lymphadenopathy and splenic involvement (Chaps. 3 to 5). Prolymphocytic leukemia and hairy cell leukemia, two uncommon B-lymphocyte malignancies, are prone to infiltrate the marrow and spleen, sometimes causing bone marrow fibrosis and massive splenomegaly (Chaps. 3 and 4).

## LYMPHOKINE-INDUCED DISORDERS

In addition to the consequences of monoclonal immunoglobulin and tumor proliferation, some lymphoid malignancies may produce cytokines that contribute to the disease morbidity. Recent work has identified several immune activation syndromes, mediated in large part as a result of unchecked inflammatory cytokines (interleukin [IL] 1, IL-6) and defects in perforin/granzyme pathways, are associated with lymphomas and infection with oncogenic herpes viruses. Hemophagocytic lymphohistiocytosis and macrophage-activating syndrome are two distinct complications arising from dysregulated effector lymphocyte-tumor interaction at the immunologic synapse and can lead to life-threatening complications if not rapidly diagnosed and treated with



immunochemotherapy.<sup>33</sup> Patients with cutaneous T-cell lymphomas have elevated plasma levels of T-helper type 2 (Th2)-associated cytokines, which may account for the relatively high incidence of eosinophilia (Chap. 14) and eosinophilic pneumonia observed in patients with this disease.<sup>34</sup> In addition, the neoplastic plasma cells in myeloma may secrete various cytokines and osteoclast-activating factors that stimulate osteoclast proliferation and activity, leading to extensive osteolysis, severe bone pain, and pathologic fractures (Chap. 18).<sup>35</sup> Dysregulated extrarenal production of calcitriol, the active metabolite of vitamin D, appears to underlie the hypercalcemia associated with Hodgkin lymphoma and other lymphomas (Chaps. 6 and 8).<sup>36</sup>

## SYSTEMIC SYMPTOMS

Large cell lymphoma, poorly differentiated lymphoma, and Hodgkin lymphoma frequently are associated with fever, night sweats, weight loss, and anorexia-cachexia (Chaps. 6, 8, and 9). Patients with lymphomas or Hodgkin lymphoma have an increased incidence of localized or disseminated herpes zoster,<sup>37</sup> and 10 percent or more of these patients may be affected at some time during the course of their illness. Pruritus is common in Hodgkin lymphoma,<sup>38</sup> and its severity parallels disease activity (Chap. 8). Systemic symptoms may be present in Hodgkin lymphoma in the absence of obvious, bulky lymph node or splenic tumors, whereas in well-differentiated small cell lymphomas, such as CLL or Waldenström macroglobulinemia, fever, night sweats, and significant weight loss are uncommon despite generalized lymphadenopathy and splenomegaly. Rather, fever in patients with CLL or macroglobulinemia usually is secondary to infectious disease (Chaps. 3 and 20).

## METABOLIC SIGNS

Lymphoid malignancies are associated with several dramatic metabolic disturbances associated with cancers (Chap. 6). Some lymphomas and lymphocytic leukemias may have a high proliferative rate, a high death fraction of cells, and, therefore, an enormous turnover of nucleoproteins, sometimes causing hyperuricemia and extreme hyperuricosuria. Highly proliferative neoplasms like Burkitt lymphoma or lymphoblastic lymphoma are particularly likely to cause an extreme degree of hyperuricemia, sometimes leading to renal failure complicating initiation of cytotoxic therapy (Chaps. 2 and 13). Also, because these and other lymphocytic malignancies are sensitive to cytotoxic drugs and glucocorticoids, cytotoxic therapy may cause a *tumor lysis syndrome*, characterized by extreme hyperuricemia, hyperuricosuria, hyperkalemia, and/or hyperphosphatemia.<sup>39,40</sup> Precipitation of uric acid in the renal tubules and collecting system can lead to acute obstructive nephropathy and renal failure unless precautions are taken, such as pretreatment with allopurinol, hydration, and alkalization of the urine.<sup>41</sup> For extreme cases, or in cases in which allopurinol cannot be administered (e.g., drug allergy), the drug rasburicase may be required for treatment of hyperuricemia (Chap. 13).<sup>42</sup>

Hypercalcemia and calciuria are common complications of myeloma because of osteolysis. Hypercalcemia also may occur during the course of lymphomas (Chap. 5) or myeloma (Chap. 18). This situation may be caused by several mechanisms, including tumor cell production of IL-1, ectopic parathyroid hormone elaboration, excessive bone resorption, and impaired bone formation.<sup>43</sup>

## EXTRANODAL INVOLVEMENT

T-cell leukemias and lymphomas, in addition to causing lymph node and spleen enlargement, may involve the skin, mediastinum, or CNS. As the name implies, cutaneous T-cell lymphomas have malignant cells

that home to the skin,<sup>44</sup> sometimes producing a severe desquamating erythroderma, as in Sézary syndrome, or small (<2-cm) subcutaneous nodules, as in primary cutaneous CD30-positive T-cell lymphoproliferative disease or anaplastic large cell lymphoma,<sup>45</sup> or a variety of nodular infiltrative lesions, as in mycosis fungoides or adult T-cell leukemia/lymphoma associated with human T-cell leukemia virus type 1 (HTLV-1; Chap. 14).<sup>46</sup> T-cell acute lymphoblastic leukemia and lymphoblastic lymphoma frequently cause mediastinal enlargement (Chap. 2). These diseases frequently involve testicles and the leptomeninges and other structures that are transverse to the subarachnoid space, such as the cranial and peripheral nerves.

B-cell lymphomas frequently may involve the salivary glands, endocrine glands, joints, heart, lung, kidney, bowel, bone, or, less frequently, other extranodal sites, such as the CNS and testes (Chap. 6). These diseases may begin as an extranodal tumor, or the tumor may develop during the course of the disease. Aggressive lymphomas, such as Burkitt lymphoma,<sup>47</sup> primary testicular lymphoma,<sup>47,48</sup> and double-hit DLBCLs (Chap. 9),<sup>49</sup> frequently involve the CNS and require upfront assessment during diagnosis and treatment with either intrathecal chemotherapy or regimens capable of crossing the blood-brain barrier that contain high-dose methotrexate. Marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) type frequently involves the stomach and salivary glands, although the disease may be encountered in any extranodal site distinguished by the presence of a columnar or cuboidal epithelium.

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## CHAPTER 2

# ACUTE LYMPHOBLASTIC LEUKEMIA

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### SUMMARY

Acute lymphoblastic leukemia (ALL) is a malignant disorder that originates in a single B- or T-lymphocyte progenitor. Proliferation and accumulation of clonal blast cells in the marrow result in suppression of hematopoiesis and, thereafter, anemia, thrombocytopenia, and neutropenia. Lymphoblasts can accumulate in various extramedullary sites, especially the meninges, gonads, thymus, liver, spleen, and lymph nodes. The disease is most common in children but can be seen in individuals of any age. ALL has many subtypes and can be classified by immunologic, cytogenetic, and molecular genetic methods. These methods can identify clinically important, biologic subtypes, requiring treatment approaches that differ in their use of specific drugs or drug combinations, dosages of drug, or duration of treatment required to achieve optimal results. For example, cases of childhood ALL having a hyperdiploid karyotype respond well to extended treatment with methotrexate and mercaptopurine, whereas adults whose leukemic cells contain the Philadelphia chromosome and *BCR-ABL1* fusion benefit from intensive treatment that includes a tyrosine kinase inhibitor and transplantation of allogeneic hematopoietic stem cells. The relative lack of therapeutic success in adult ALL is partly related to a high frequency of cases having unfavorable genetic abnormalities and partly related to poor tolerance for intensive treatment. Nearly 90 percent of children and 40 percent of adults can expect long-term, leukemia-free survival—and probable cure—with contemporary treatment. Novel immunotherapeutic approaches are under development. Currently, emphasis is placed not only on improving the cure rate but also on improving quality of life by preventing acute and late treatment-related complications, such as second malignancies, cardiotoxicity, and endocrinopathy.

### DEFINITION AND HISTORY

Acute lymphoblastic leukemia (ALL) is a neoplastic disease that results from multistep somatic mutations in a single lymphoid progenitor cell at one of several discrete stages of development. The immunophenotype of leukemic cells at diagnosis reflects the level of differentiation achieved by the dominant clone. The clonal origin of ALL has been established by cytogenetic analysis, by analysis of restriction fragments in female

patients who are heterozygous for polymorphic X chromosome-linked genes, and by analysis of rearrangements of T-cell receptor or immunoglobulin genes. Leukemic cells divide more slowly and require more time to synthesize DNA than do normal hematopoietic counterparts. However, leukemic cells accumulate relentlessly because of their altered response to growth and death signals.<sup>1,2</sup> They compete successfully with normal hematopoietic cells, resulting in anemia, thrombocytopenia, and neutropenia. At diagnosis, leukemic cells not only have replaced normal marrow cells but also have disseminated to various extramedullary sites.

Velpeau<sup>3</sup> is generally credited with the earliest report, in 1827, of leukemia. Virchow,<sup>4</sup> Bennett,<sup>5</sup> and Craigie<sup>6</sup> recognized the condition as a distinct entity by 1845. In 1847, Virchow coined the terms *weisses blut* and, later, *leucaemie*, applying them to two distinct types of the disease—splenic and lymphatic—that could be distinguished from each other based on splenomegaly and enlarged lymph nodes and on the morphologic similarities of the leukemic cells to those normally found in these organs.<sup>7</sup> Ehrlich's introduction of staining methods in 1891 allowed further distinction of leukemia subtypes.<sup>8</sup> By 1913, leukemia could be classified as acute or chronic and as lymphatic or myelogenous.<sup>9</sup> The greater prevalence of ALL in children, especially those ages 1 to 5 years, was recognized in 1917.<sup>10</sup>

Shortly after leukemia was recognized as a discrete disease entity, physicians began using chemicals as palliative therapy. The first advance was the use of a four-amino analogue of folic acid (aminopterin), prompted by Farber's observation that folic acid appeared to accelerate the proliferation of leukemic cells. Strikingly, for the first time, complete clinical and hematologic remissions that lasted for several months were seen in children.<sup>11</sup> A year after the report of aminopterin-induced clinical remissions, a newly isolated adrenocorticotrophic hormone was reported to induce prompt, though brief, remissions in patients with leukemia.<sup>12</sup> Almost concurrently, Elion and colleagues synthesized antimetabolites that interfere with synthesis of purines and pyrimidines.<sup>13</sup> Their findings led to the introduction of mercaptopurine, 6-thioguanine, and allopurinol into clinical use. From 1950 to 1960, many new antileukemic agents were introduced, and occasional cures were seen. Pinkel and colleagues at St. Jude Children's Research Hospital, in 1962, devised a "total therapy" approach, consisting of four treatment phases: remission induction; intensification or consolidation; therapy for subclinical CNS leukemia (or preventive meningeal treatment); and prolonged continuation therapy.<sup>14</sup> By the early 1970s, as many as 50 percent of children achieved long-term event-free survival (EFS) using this innovative strategy. During the same period, a better understanding of the genetics of human histocompatibility and wider use of human leukocyte antigen (HLA) typing culminated in the successful use of hematopoietic stem cell transplantation for treatment of patients in whom leukemia relapsed. In the early 1980s, Riehm and coworkers introduced a so-called reinduction or delayed intensification treatment during early continuation therapy, consisting mainly of repetition of the initial remission induction and early intensification phases, and further improved the EFS to approximately 70 percent.<sup>15</sup> Parallel to advances in treatment has been the improved understanding of the biology of ALL. The recognition of ALL as a heterogeneous group of diseases—clinically, immunologically, and genetically<sup>16</sup>—set the stage for risk-directed therapy.<sup>16a</sup>

Treatment of ALL has progressed incrementally, beginning with the development of effective therapy for CNS disease, followed by intensification of early treatment, especially for patients at high risk of relapse. The current cure rates of nearly 90 percent for children (Fig. 2-1) and 40 percent for adults attest to the steady progress made in treating this disease.<sup>17,18</sup> Rapid evolution and convergence of multiple genome-wide platforms to identify the total complement of genetic and epigenetic alterations almost certainly will lead to the identification of

**Acronyms and Abbreviations:** ALL, acute lymphoblastic leukemia; ARID5B, AT-rich interactive domain 5b; *ATM*, ataxia-telangiectasia mutated gene; CD, cluster of differentiation; CNAs, copy number abnormalities; CSF, cerebrospinal fluid; EFS, event-free survival; FISH, fluorescence *in situ* hybridization; HLA, human leukocyte antigen; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; SEER, Surveillance, Epidemiology, and End Results; SNP, single nucleotide polymorphism.

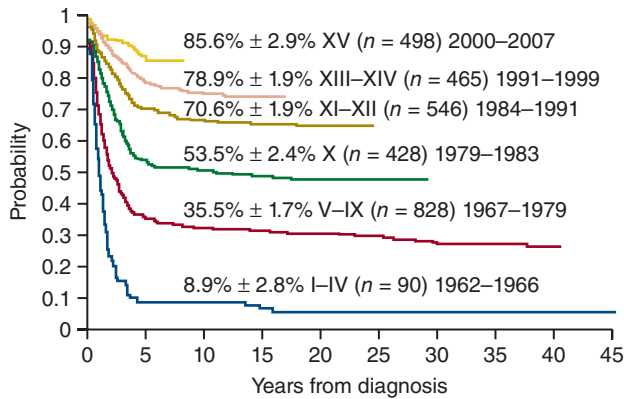


**Figure 2-1.** Kaplan-Meier analysis of event-free survival for 2855 children with ALL treated in 15 consecutive total-therapy studies at St. Jude Children's Research Hospital. Early intensification of systemic and intrathecal chemotherapy with a risk assignment based on sequential measurements of minimal residual disease in the 2000s has boosted the event-free survival estimate to 85.6 percent ± 2.9 percent (SE). (Data from CH Pui and are unpublished.)

new targets for specific treatment.<sup>19,20</sup> A clear advance was the development of imatinib mesylate and dasatinib, which target leukemias with the *BCR-ABL1* fusion.<sup>21</sup>

## ETIOLOGY AND PATHOGENESIS

Initiation and progression of ALL are driven by successive mutations that alter cellular functions, including an enhanced ability of self-renewal, a subversion of control of normal proliferation, a block in differentiation, and an increased resistance to death signals (apoptosis).<sup>1,2</sup> Familial disorders of DNA repair may play a role. Environmental agents, such as ionizing radiation and chemical mutagens, have been implicated in the induction of ALL in some patients. However, in most cases, no etiologic factors are discernible. In the favored theory, leukemogenesis reflects the interaction between host pharmacogenetics (susceptibility) and environmental factors, a model that requires confirmation in well-designed population and molecular epidemiologic studies.

## INCIDENCE

The American Cancer Society estimated that in the United States there would be approximately 6020 new cases of ALL in 2014 (3140 in males and 2880 in females) and approximately 1440 deaths from ALL (810 in males and 630 in females).<sup>22</sup> Most cases of ALL occur in children, but most deaths from ALL (approximately four of five) occur in adults.

The age-adjusted incidence rate of ALL was 1.6 per 100,000 males and 1.2 for females per year in the United States, based on cases diagnosed in 1975 to 2010 from 17 Surveillance, Epidemiology, and End Results (SEER) geographic areas.<sup>23</sup> The risk for developing ALL is highest in children younger than 5 years of age. The risk then declines slowly until the mid-20s and begins to rise again slowly after age 50. The incidence is 7.9 per 100,000 children 1 to 4 years old and 1.2 for those older than age 60 years. Only 20 percent of adult acute leukemias are ALL, but about one-third of ALL cases are in adults. The average person's lifetime risk of developing ALL is less than one in 750. The risk is slightly higher in males than in females and higher in whites than in African Americans (Fig. 2-2).<sup>23</sup> The median age at diagnosis for ALL is 13 years, and approximately 61 percent of individuals are diagnosed before the age of 20 years; however, because of the bimodal peak in incidence, the age of

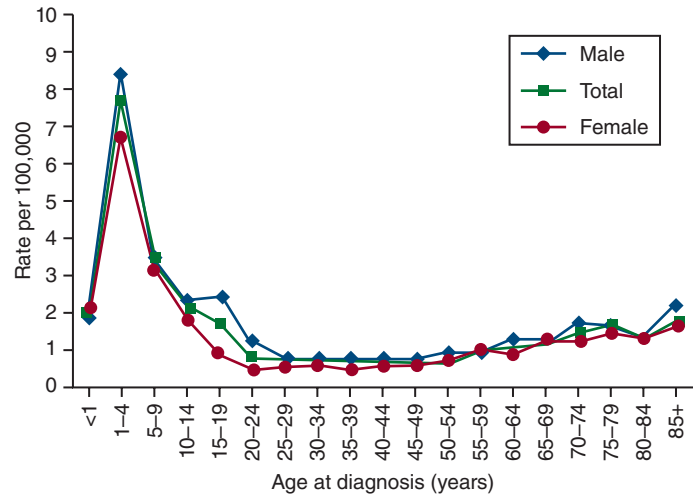


**Figure 2-2.** Age-specific incidence rates for acute lymphoblastic leukemia by sex. (Data from SEER Cancer Statistics Review, 1975–2010, National Cancer Institute, Bethesda, MD. [http://seer.cancer.gov/csr/1975\\_2010](http://seer.cancer.gov/csr/1975_2010). Accessed July 4, 2014.)

13 years is mathematically correct but medically nearly useless. ALL is the most common malignancy diagnosed in patients younger than age 15 years, accounting for 23 percent of all cancers and 76 percent of all leukemias in this age group.

The sharp incidence peak of ALL during early childhood has been observed only since the 1930s in the United Kingdom and the United States.<sup>24</sup> In the United States, the peak first appeared in children of European descent and subsequently was seen in children of African descent in the 1960s. The age peak is absent in many developing or underdeveloped countries, suggesting a leukemogenic contribution from factors associated with industrialization. Except for a slight predominance for females in infancy, ALL affects males of European descent more often than females in all age groups (Fig. 2-2). The frequency distribution is similar among those of African descent. In most age groups, the incidence of ALL is higher in those of European descent than in those of African descent, especially among children ages 2 to 3 years.

The incidence of ALL differs substantially in different geographic areas. Rates are higher among populations in northern and western Europe, North America, and Oceania, with lower rates in Asian and African populations.<sup>25</sup> In Europe, the highest rates of ALL among males are found in Spain and the highest rates among females in Denmark. In the United States, the highest rates for both sexes are among Latinos in Los Angeles.

## RISK FACTORS

### Genetic Syndromes

The precise pathogenetic events leading to the development of ALL are unknown. Only a minority (5 percent) of cases are associated with inherited, predisposing genetic syndromes. Children with Down syndrome have a 10 to 30 times greater risk of leukemia; acute megakaryoblastic leukemia predominates in those patients younger than age 3 years, and ALL is predominant in older age groups. ALL in patients with Down syndrome is a heterogeneous disorder, comprising subtypes with the same well-recognized genetic abnormalities found in the general population, such as hyperdiploidy greater than 50 and *t*(12;21)[*ETV6-RUNX1*], plus those more commonly associated with Down syndrome, such as *+X*, *del*(9), and *CEBPD* rearrangement.<sup>26,27</sup> Studies show that *P2RY8-CRLF2* fusion and activating *JAK* mutations together contribute



to leukemogenesis in approximately half of the cases of Down syndrome patients with ALL.<sup>28,29</sup> Almost all ALL patients with Down syndrome have a deletion of IKZF1.<sup>30</sup> Autosomal recessive genetic diseases associated with increased chromosomal fragility and a predisposition to ALL include ataxia-telangiectasia, Nijmegen breakage syndrome, and Bloom syndrome.<sup>31</sup> Patients with ataxia-telangiectasia have a 70 times greater risk of leukemia and a 250 times greater risk of lymphoma, particularly of the T-cell phenotype.<sup>32</sup> The causative gene, termed *ATM* (ataxia-telangiectasia mutated), encodes a protein involved in DNA repair, regulation of cell proliferation, and apoptosis. Laboratory studies supporting the diagnosis of ataxia-telangiectasia include an elevated serum concentration of  $\alpha$ -fetoprotein, presence of characteristic chromosomal aberrations, absent or reduced intranuclear serine protein kinase ATM, and increased *in vitro* radiosensitivity. A high prevalence of germline truncating and missense *ATM* gene alterations in children with sporadic T-cell ALL suggests a pathogenetic role of *ATM* in lymphoid malignancies. Although impaired immune surveillance contributes to the increased risk of Epstein-Barr-virus-related malignancies in patients with acquired immunodeficiencies, no compelling evidence indicates defective immunity contributes to the predisposition to ALL in patients with ataxia-telangiectasia or other congenital immunodeficiency syndromes. Genome-wide association studies have identified common allelic variants in four genes (*IKZF1*, *ARID5B*, *CEBPE*, and *CDKN2a*) that are consistently associated with childhood ALL.<sup>33–35</sup> These genes are key regulators of blood cell development, and acquired mutations of each are also detected in ALL cases. Thus, the risk of childhood ALL may be influenced by coinheritance of multiple low-risk variants. Inherited allelic variation may also affect response to treatment.<sup>36</sup>

### Environmental Factors

*In utero* (but not postnatal) exposure to diagnostic x-rays confers a slightly increased risk of ALL, which correlates positively with the number of exposures.<sup>37</sup> The evidence is weak for an association between the development of ALL and nuclear fallout; exposure to occupational, natural terrestrial, or cosmic ionizing radiation; or paternal radiation exposure prior to conception. There has been concern that exposure to low-energy electromagnetic fields produced by a residential power supply may be associated with the development of childhood ALL. Case-control studies suggested a slightly increased risk of leukemia at very high levels of exposure; assuming the association is real, only approximately 1 percent of leukemias could be attributed to the exposure.<sup>38,39</sup> Pesticide exposure (occupational or home use) and parental cigarette smoking before or during pregnancy, administration of vitamin K to neonates, maternal alcohol consumption during pregnancy, and increased consumption of dietary nitrites have each been suggested causes. However, each of these associations is controversial, and most have been refuted after careful, controlled investigation. High birth weight is associated with an increased risk of leukemia before the age of 5 years with fair consistency,<sup>40</sup> and the birth weight is likely a marker for an endogenous factor, such as insulin-like growth factor.

### Host Pharmacogenetics

Subtle genetic polymorphisms of xenobiotic-metabolizing enzymes, DNA repair pathways, and cell-cycle checkpoint functions might interact with environmental, dietary, maternal, and other external factors to affect the development of ALL.<sup>2,41</sup> Although the number of investigations and sample sizes are limited, data exist to support a causal role for polymorphisms in genes encoding detoxifying enzymes (e.g., glutathione S-transferase, nicotinamide adenine dinucleotide phosphate [NAD(P)H]: quinone oxidoreductase), folate-metabolizing enzymes (serine hydroxymethyltransferase and thymidylate synthase), cytochrome P450, methylenetetrahydrofolate reductase, and cell-cycle inhibitors in the development

of adult and childhood ALL.<sup>42,43</sup> However, all these associations must be confirmed by larger studies with careful attention to ethnic and geographic diversity in the frequency of polymorphisms. Using genome-wide analysis, germline single nucleotide polymorphisms (SNPs) of AT-rich interactive domain 5b (*ARID5B*) gene have been associated with childhood hyperdiploid B-cell precursor ALL,<sup>44</sup> a clear example of host genetic variations affecting the susceptibility to the development of childhood ALL.

### Development of Acute Lymphoblastic Leukemia In Utero

Retrospective identification of leukemia-specific fusion genes (e.g., *KMT2A/AFF1* [also known as *MLL-AF4*] and *ETV6-RUNX1* [also known as *TEL-AML1*]), hyperdiploidy, or clonotypic rearrangements of immunoglobulin or T-cell receptor loci in archived neonatal blood spots (Guthrie cards) and development of concordant leukemia in identical twins clearly indicate some leukemias have a prenatal origin.<sup>45,46</sup> In identical twins with the t(4;11)/*KMT2A/AFF1*, the concordance rate is nearly 100 percent, and the latency in the time of occurrence in the two twins is short (a few weeks to a few months). These findings suggest this fusion gene alone either is leukemogenic or requires only a small number of cooperative mutations to cause leukemia. By contrast, the lower concordance rate in twins with the *ETV6-RUNX1* fusion or T-cell phenotype and the longer postnatal latency period suggest additional postnatal events are required for leukemic transformation in these subtypes.<sup>45</sup> This theory is supported by the identification of rare cells expressing *ETV6-RUNX1* fusion transcripts in approximately 1 percent of cord blood samples from newborns, a frequency 100 times higher than the incidence of ALL defined by this fusion transcript.<sup>45</sup> The presence of a preleukemic clone with the *ETV6-RUNX1* has been established.<sup>47</sup> Hyperdiploid ALL, another common subtype of childhood ALL, also appears to arise before birth but requires postnatal events for full malignant transformation.<sup>46</sup> The observations of a peak age of development of childhood ALL of 2 to 5 years, an association of industrialization and modern or affluent societies with increased prevalence of ALL, and the occasional clustering of childhood leukemia cases have fueled two parallel infection-based hypotheses to account for postnatal events. The “delayed infection” hypothesis suggests that some susceptible individuals with a prenatally acquired preleukemic clone had low or no exposure to common infections early in life because they lived in an affluent hygienic environments.<sup>45</sup> Such infectious insulation predisposes the immune system of these individuals to aberrant or pathologic responses after subsequent or delayed exposure to common infections at an age commensurate with increased lymphoid cell proliferation. The “population-mixing” hypothesis predicts that clusters of childhood ALL result from exposure of susceptible (nonimmune) individuals to common but fairly nonpathologic infections after population mixing with carriers.<sup>48</sup> However, clearly not all childhood cases develop *in utero*. For example, t(1;19)/*TCF3-PBX1* (also known as *E2A-PBX1*) ALL appears to have a postnatal origin in most cases.<sup>49</sup> Cases of adult ALL most certainly arise over a protracted time.

### ACQUIRED GENETIC CHANGES

Acquired genetic abnormalities are a hallmark of ALL; 80 percent of all cases have recurring cytogenetic or molecular lesions with prognostic and therapeutic relevance (Table 2–1).<sup>2,19,41</sup> Chromosomal changes include abnormalities in the number (ploidy) and structure of chromosomes.<sup>50–52</sup> The latter comprise translocations (the most frequent abnormality), inversions, deletions, point mutations, and amplifications. Although the frequency of particular genetic subtypes differs between childhood and adult cases, the general mechanisms underlying the induction are similar. Mechanisms include aberrant expression of oncoproteins, loss of



**TABLE 2-1.** Frequencies of Common Genetic Aberrations in Childhood and Adult Acute Lymphoblastic Leukemia

| Abnormality                                              | Children (%)            | Adults (%) |
|----------------------------------------------------------|-------------------------|------------|
| Hyperdiploidy (>50 chromosomes)                          | 23–29                   | 6–7        |
| Hypodiploidy (<45 chromosomes)                           | 1                       | 2          |
| t(1;19)(q23;p13.3) [TCF3-PBX1]                           | 4 in white, 12 in black | 2–3        |
| t(9;22)(q34;q11.2) [BCR-ABL1]                            | 2–3                     | 25–30      |
| t(4;11)(q21;q23) [MLL-AF4]                               | 2                       | 3–7        |
| t(8;14)(q23;q32.3)                                       | 2                       | 4          |
| t(12;21)(p13;q22) [ETV6-RUNX1]                           | 20–25                   | 0–3        |
| NOTCH1 mutations*                                        | 7                       | 15         |
| HOX11L2 overexpression*                                  | 20                      | 13         |
| LYL1 overexpression*                                     | 9                       | 15         |
| TAL1 overexpression*                                     | 15                      | 3          |
| HOX11 overexpression*                                    | 7                       | 30         |
| MLL-ENL fusion                                           | 2                       | 3          |
| Abnormal 9p                                              | 7–11                    | 6–30       |
| Abnormal 12p                                             | 7–9                     | 4–6        |
| del(7p)/del(7q)/monosomy 7                               | 4                       | 6–11       |
| +8                                                       | 2                       | 10–12      |
| Intrachromosomal amplification of chromosome 21 (iAMP21) | 2                       | ?          |
| Ph-like genotype                                         | 10                      | 27         |

\*Abnormalities found in T-cell acute lymphoblastic leukemia (ALL).

tumor-suppressor genes, and chromosomal translocations that generate fusion genes encoding transcription factors or active kinases.

Primary genetic rearrangement by itself is insufficient to induce overt leukemia. Cooperative mutations are necessary for leukemic transformation and include genetic and epigenetic changes in key growth regulatory pathways.<sup>19,20</sup> The candidate gene approach has identified deletion of the *CDKN2A/CDKN2B* tumor-suppressor locus<sup>53</sup> and mutations of *NOTCH1* in T-cell ALL.<sup>54</sup> Current searches applying genome-wide microarray and high-throughput sequencing methodologies have identified a high frequency of common genetic alterations in both B-cell precursor ALL and T-cell ALL. Using SNP microarray, a mean of 6.46 DNA copy number abnormalities (CNAs) per case was identified, suggesting that gross genomic instability is not a feature for most ALL cases.<sup>55</sup> There was a wide variation in the number of CNAs across leukemic subtypes. Interestingly, infant ALL cases with *MLL* rearrangement had less

than one CNA per case, suggesting that few additional genetic lesions are required for leukemogenesis in these cases. By contrast, *ETV6-RUNX1* and *BCR-ABL1* cases had more than six CNAs per case, with some having more than 20 lesions, a finding consistent with the concept that, although the initiating events may occur early in childhood, additional lesions are required for subsequent development of ALL. More than 40 percent of B-cell precursor ALL cases had mutations in genes encoding regulators of normal lymphoid development.<sup>55</sup> The most frequent target was the lymphoid transcription factor *PAX5* (mutated in approximately 32 percent of cases), which encodes a paired-domain protein required for the pro-B-cell to pre-B-cell transition and B-lineage fidelity. The second most frequently involved gene was *IKZF1* (mutated in almost 28 percent of the cases), encoding the IKAROS zinc finger DNA-binding protein that is required for the earliest lymphoid differentiation. *IKZF1* was deleted in the vast majority of cases of *BCR-ABL1* ALL cases as well as chronic myeloid leukemia in lymphoid blast crisis (but not chronic phase).<sup>56</sup> Approximately half of *BCR-ABL1* ALL cases also had deletions of *CDKN2A/B* and *PAX5*. This finding further supports the concept that multiple signaling pathways need to be disrupted to induce leukemia. A subgroup of ALL with very poor outcome was strongly associated with the presence of *IKZF1* deletions.<sup>57,58</sup> Together, these findings suggest that *IKZF1* directly contributes to treatment resistance in ALL.

*BCR-ABL1*-like B-cell ALL lacks the *BCR-ABL1* fusion or t(9;22) by cytogenetic, fluorescence *in situ* hybridization (FISH), or molecular analyses, but it shares the same gene-expression profile with typical *BCR-ABL1*-positive ALL.<sup>59,59a–59c</sup> In half of these cases, the *CRLF2* gene is involved in a cryptic translocation with the *IGH* gene or is fused to the *P2RY8* gene; both rearrangements lead to overexpression of *CRLF2*.<sup>29,60</sup> Mutations in *JAK2* or *JAK1* are detected in 30 to 40 percent of these cases, and many of the remaining have activating mutations in cytokine receptor and kinase signaling pathways.<sup>30</sup> Microarrays and genomic DNA sequencing identified monoallelic deletion of the *PAX5* gene at chromosome band 9p13.2 in 28 percent of ALL patients with cryptic or larger deletions on 9p.<sup>55</sup>

Gene-expression profiling with DNA microarrays allows nearly all T-cell cases to be grouped according to multistep oncogenic pathways.<sup>61</sup> Gene-expression studies also show that overexpression of *FLT3*, a receptor tyrosine kinase important for development of hematopoietic stem cells, is a secondary event in almost all cases with either *MLL* rearrangements or hyperdiploidy.<sup>62</sup> The finding has provided an impetus for clinical testing of *FLT3* inhibitors in ALL. Other genome-wide interrogations of both leukemia cells and germline tissues have identified other genetic variations with prognostic or therapeutic relevance and may lead to the development of specific treatment.<sup>17,36</sup>

Epigenetic changes, including hypermethylation and silencing of tumor-suppressor genes and hypomethylation of oncogenes and abnormalities in posttranscriptional control mechanisms, such as those involving microRNA, are common findings in cancer. These changes are reversible and do not alter the DNA sequence, yet they can alter gene expression in subtle ways that encourage malignant transformation and progression. The analysis of epigenetic alterations has begun to apply to the development of new biomarkers for risk assignment or disease monitoring and to the design of alternative treatment in ALL.<sup>63</sup> Evidence indicates that the methylation of multiple genes in ALL is associated with a worse outcome. Surprisingly, methylation of genes was as prominent in childhood as in adult ALL. The differences in the response of children and adults appear not to be related to quantitative methylation but to the specific genes and the specific pathways deactivated. Preliminary studies of hypomethylating agents (e.g., azacitidine and decitabine) are being tested in patients refractory or resistant to current drug programs.<sup>64</sup>

## CLINICAL FEATURES

### SIGNS AND SYMPTOMS

The clinical presentation of ALL is highly variable. Symptoms may appear insidiously or acutely. The presenting features generally reflect the degree of marrow failure and the extent of extramedullary spread (Table 2-2).<sup>18,65-67</sup> Approximately half of patients present with fever, which can be caused by either neutropenia-induced infection or leukemia-released cytokines (e.g., interleukin-1, interleukin-6, and tumor necrosis factor) released from leukemia cells. In these patients, fever resolves within 72 hours after the start of antileukemia therapy.

Fatigue and lethargy are common manifestations of anemia in patients with ALL. In older patients, anemia-related dyspnea and lightheadedness may be the dominant presenting features. More than 25 percent of patients, especially young children, may have a limp from bone pain or arthralgia; an unwillingness to walk because of leukemic infiltration of the periosteum, bone, or joint; or because of expansion

of the marrow cavity by leukemia cells. Children with prominent bone pain often have nearly normal blood counts, which can contribute to delayed diagnosis. In a small proportion of patients, marrow necrosis can result in severe bone pain and tenderness, fever, and a very high level of serum lactate dehydrogenase.<sup>68,69</sup> Arthralgia and bone pain are less severe in adults. Less common signs and symptoms include headache, vomiting, altered mental function, oliguria, and anuria. Occasionally, patients present with a life-threatening infection or bleeding (e.g., intracranial hematoma). Intracranial hemorrhage occurs mainly in patients with an initial leukocyte count greater than  $400 \times 10^9/L$ .<sup>70</sup> Very rarely, ALL produces no signs or symptoms and is detected during routine examination.

### PHYSICAL FINDINGS

Among frequent findings are pallor, petechiae, and ecchymosis in the skin and mucous membranes and bone tenderness as a result of leukemic infiltration or hemorrhage that stretches the periosteum. Liver, spleen, and lymph nodes are the most common sites of extramedullary involvement, and the degree of organomegaly is more pronounced in children than in adults. An anterior mediastinal (thymic) mass is present in 8 to 10 percent of childhood cases and in 15 percent of adult cases (Fig. 2-3). A bulky, anterior mediastinal mass can compress the great vessels and trachea and possibly lead to superior vena cava syndrome. Patients with this syndrome present with cough, dyspnea, orthopnea, stridor, cyanosis, dysphagia, facial edema, increased intracranial pressure, and sometimes syncope. Painless enlargement of the scrotum can be a sign of testicular leukemia cell infiltration or hydrocele, the latter resulting from lymphatic obstruction. Both conditions can be readily diagnosed by ultrasonography. Overt testicular disease is relatively rare, is generally seen in infants or adolescents with T-cell leukemia and/or hyperleukocytosis, and does not require radiation therapy.<sup>71</sup> Other uncommon presenting features include ocular involvement (leukemic infiltration of the orbit, optic nerve, retina, iris, cornea, or conjunctiva), subcutaneous nodules (leukemia cutis), enlarged salivary

**TABLE 2-2.** Presenting Clinical Features in Children and Adults with Acute Lymphoblastic Leukemia

| Feature                  | Children (%) | Adult (%) |
|--------------------------|--------------|-----------|
| Age (years)              |              |           |
| <1                       | 2            | —         |
| 1–9                      | 72–78        | —         |
| 10–19                    | 20–26        | —         |
| 20–39                    | —            | 40        |
| 40–59                    | —            | 40        |
| ≥60                      | —            | 20        |
| Male                     | 56–57        | 62        |
| Symptoms                 |              |           |
| Fever                    | 57           | 33–56     |
| Fatigue                  | 50           | Common    |
| Bleeding                 | 43           | 33        |
| Bone or joint pain       | 25           | 25        |
| Lymphadenopathy          |              |           |
| None                     | 30           | 51        |
| Marked (>3 cm)           | 15           | 11        |
| Hepatomegaly             |              |           |
| None                     | 34           | 65        |
| Marked (below umbilicus) | 17           | Rare      |
| Splenomegaly             |              |           |
| None                     | 41           | 56        |
| Marked (below umbilicus) | 17           | Uncommon  |
| Mediastinal mass         | 8–10         | 15        |
| CNS leukemia             | 3            | 8         |
| Testicular leukemia      | 1            | 0.3       |

Data from Pui CH: *Acute lymphoblastic leukemia*, in *Childhood Leukemias*, 2nd ed, edited by CH Pui, p 439. Cambridge University Press, New York, 2006; and Larson RA, Dodge RK, Burns CP, et al: A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. *Blood* 85:2025, 1995.



**Figure 2-3.** Chest radiograph of a 12-year-old black male with T-cell acute lymphoblastic leukemia (ALL) and an anterior mediastinal mass.

glands (Mikulicz syndrome), cranial nerve palsy, and priapism (resulting from leukostasis of the corpora cavernosa and dorsal veins or sacral nerve involvement). Epidural spinal cord compression at presentation is a rare but serious finding that requires immediate treatment to prevent permanent paraparesis or paraplegia. In some pediatric patients, infiltration of tonsils, adenoids, appendix, or mesenteric lymph nodes leads to surgical intervention before leukemia is diagnosed.

## LABORATORY FEATURES

Anemia, neutropenia, and thrombocytopenia are common in patients with newly diagnosed ALL. The severity reflects the degree of marrow replacement by leukemic lymphoblasts (Table 2-3).<sup>18,65-67</sup> Presenting leukocyte counts range widely, from 0.1 to  $1500 \times 10^9/L$  (median:  $10-12 \times 10^9/L$ ). Hyperleukocytosis ( $>100 \times 10^9$  white cells/L) occurs in 11 to 13 percent of white children, but occurs more often in black children (23 percent) and in adults (16 percent) because they are more likely to have T-cell ALL. Profound neutropenia ( $<0.5 \times 10^9/L$ ) is found in 20 to 40 percent of patients, elevating their risk for infection. Approximately 90 percent of patients have circulating leukemic blast cells at diagnosis. Hypereosinophilia, generally reactive, may precede the diagnosis of ALL by several months. Some patients, mostly male, have ALL with the t(5;14)(q31;q32) chromosomal abnormality and a hypereosinophilic syndrome (pulmonary infiltration, cardiomegaly, and congestive heart failure). These patients often do not have circulating leukemic blasts or other cytopenias and have a relatively low percentage of blasts in the marrow.<sup>72</sup> Activation of the interleukin-3 gene on chromosome 5 by the enhancer element of the immunoglobulin heavy-chain gene on chromosome 14 is thought to contribute to leukemogenesis and the associated eosinophilia in these cases.<sup>72</sup> In patients with anemia, hemoglobin levels are lower in younger children. Occasionally, a child with ALL has a hemoglobin level as low as 1 g/dL.

Decreased platelet counts are common at diagnosis (median:  $48-52 \times 10^9/L$ ). Severe bleeding is uncommon, even when platelet counts are as low as  $20 \times 10^9/L$ , provided infection and fever are absent.<sup>73,74</sup> Occasional patients, principally male, present with thrombocytosis ( $>400 \times 10^9/L$ ). Pancytopenia followed by a period of spontaneous hematopoietic recovery may precede the diagnosis of ALL in rare cases.<sup>75</sup> Coagulopathy, usually mild, can be seen in 3 to 5 percent of patients, most of whom have T-cell ALL, and is only rarely associated with clinical bleeding.<sup>76</sup> The level of serum lactate dehydrogenase is increased in most patients with ALL and is well correlated with tumor burden. Increased levels of serum uric acid are common in patients with a large leukemia cell burden, reflecting an increased rate of purine catabolism. Leukemic infiltration of the kidneys can lead to increased levels of creatinine, urea nitrogen, uric acid, and phosphorus. Rarely, patients present with hypercalcemia resulting from release of parathyroid hormone-like protein from lymphoblasts and leukemic infiltration of bone. The t(17;19)(q22;p13.3) with E2A-HLF fusion, found in 0.5 percent of B-cell precursor ALL, is associated with adolescent age group, disseminated coagulopathy, hypercalcemia, and dismal prognosis.<sup>77</sup> Liver dysfunction as a result of leukemic infiltration occurs in 10 to 20 percent of patients and is usually mild. However, recognition of carriers of hepatitis B virus is important because prompt lamivudine therapy can prevent serious complications from virus reactivation during immunosuppressive treatment.<sup>78</sup>

Chest radiography is needed to detect enlargement of the thymus or mediastinal nodes and pleural effusions (Fig. 2-3). Although bony abnormalities, such as metaphyseal banding, periosteal reactions, osteolysis, osteosclerosis, and osteopenia, can be found in 50 percent of patients, especially children with low leukocyte counts at presentation, skeletal films are not necessary for management. Magnetic resonance

**TABLE 2-3.** Presenting Laboratory Features in Children and Adults with Acute Lymphoblastic Leukemia

| Feature                               | Percent of Total          |            |
|---------------------------------------|---------------------------|------------|
|                                       | Children, White/Black (%) | Adults (%) |
| Cell lineage                          |                           |            |
| T cell                                | 15/24                     | 25         |
| B-cell precursor                      | 85/76                     | 75         |
| Leukocyte count ( $\times 10^9/L$ )   |                           |            |
| <10                                   | 47-49/34                  | 41         |
| 10-49                                 | 28-31/29                  | 31         |
| 50-99                                 | 8-12/14                   | 12         |
| >100                                  | 11-13/23                  | 16         |
| Hemoglobin concentration (g/dL)       |                           |            |
| <8                                    | 48/58                     | 28         |
| 8-10                                  | 24/22                     | 26         |
| >10                                   | 28/20                     | 46         |
| Platelet count ( $\times 10^9/L$ )    |                           |            |
| <50                                   | 46/40                     | 52         |
| 50-100                                | 23/20                     | 22         |
| >100                                  | 31/40                     | 26         |
| CNS status*                           |                           |            |
| CNS1                                  | 67-79/60                  | 92-95      |
| CNS2                                  | 5-24/27                   | ?          |
| CNS3                                  | 3/3                       | 5-8        |
| Traumatic lumbar puncture with blasts | 6-7/10                    | ?          |
| Leukemic blasts in marrow (%)         |                           |            |
| <90                                   | 33/46                     | 29         |
| >90                                   | 67/54                     | 71         |
| Leukemic blasts in blood              |                           |            |
| Present                               | 87/90                     | 92         |
| Absent                                | 13/10                     | 8          |

\*CNS1, no blast cells in cerebrospinal fluid sample; CNS2,  $<5$  leukocytes/ $\mu L$  with blast cells in a nontraumatic sample; CNS3,  $\geq 5$  leukocytes/ $\mu L$  with blast cells in a nontraumatic sample or the presence of a cranial nerve palsy; and traumatic lumbar puncture with blasts ( $\geq 10$  erythrocytes/ $\mu L$  with blasts). Data on CNS2 and traumatic lumbar puncture with blasts are not available in adults.

Data from Pui CH: *Acute lymphoblastic leukemia*, in *Childhood Leukemias*, 2nd ed, edited by CH Pui, p 439. Cambridge University Press, New York, 2006; and Larson RA, Dodge RK, Burns CP, et al: A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. *Blood* 85:2025, 1995.

imaging (MRI) is useful in patients with suspected vertebral collapse or meningeal or nerve root involvement.

Examination of the cerebrospinal fluid (CSF) is an essential diagnostic procedure.<sup>78a</sup> Leukemic blasts can be identified in as many as one-third of pediatric patients and approximately 5 percent of adult patients at diagnosis of ALL; most of these patients lack neurologic symptoms.<sup>79,80</sup> Traditionally, CNS leukemia is defined by the presence



of at least 5 leukocytes/ $\mu\text{L}$  of CSF (with leukemic blast cells apparent in a cytocentrifuged sample) or by the presence of cranial nerve palsies. However, with the omission of prophylactic cranial irradiation in contemporary clinical trials, the presence of any leukemic blast cells in the CSF is associated with increased risk of CNS relapse and is an indication to intensify intrathecal therapy.<sup>80</sup> Different opinions exist regarding when the first lumbar puncture should be performed. Most protocols now require the procedure at diagnosis and instill the first dose of chemotherapeutic agents intrathecally. Contamination of the CSF by leukemia cells as a result of traumatic lumbar puncture at diagnosis is associated with an inferior treatment outcome in children with ALL.<sup>81</sup> The risk of traumatic lumbar puncture can be decreased by administering platelet transfusions to thrombocytopenic patients and by having the most experienced clinician perform the procedure.

## DIAGNOSIS AND CELL CLASSIFICATION

Examination of a marrow aspirate is recommended for diagnosis of ALL because as many as 10 percent of patients lack circulating blasts at the time of diagnosis and because high concentrations of marrow cells are better than blood cells for genetic studies. Fibrosis or tightly packed marrow can lead to difficulties with marrow aspiration that necessitate biopsy. In patients with marrow necrosis, multiple marrow aspirations are sometimes needed to obtain diagnostic tissue.

## Morphologic and Cytochemical Analysis

Diagnosis of ALL begins with morphologic analysis of Romanowsky stained (Wright-Giemsa or May-Grünwald-Giemsa) marrow films. Lymphoblasts tend to be relatively small (ranging from the same size to twice the size of small lymphocytes), with scanty, often light-blue cytoplasm; a round or slightly indented nucleus; fine to slightly coarse and clumped chromatin; and inconspicuous nucleoli (Fig. 2-4A). In some cases, the lymphoblasts are large, with prominent nucleoli, moderate amounts of cytoplasm, admixed with smaller blasts (Fig. 2-4B). Cytoplasmic granules are found in the lymphoblasts of some patients with ALL (Fig. 2-4C). The granules usually are amphophilic (and stain fuchsia), readily distinguishable from primary myeloid granules (which stain deep purple), and demonstrated to be mitochondria by electron microscopy. B-cell blasts in Burkitt-type ALL are characterized by intensely basophilic cytoplasm, prominent nucleoli, and cytoplasmic vacuolation (Fig. 2-4D).

Cytochemical stains (Sudan black stain and the stains for myeloperoxidase and the nonspecific esterases) distinguish ALL from acute myeloid leukemia but are now less commonly used for diagnosis than immunophenotyping.

## Immunologic Classification

Immunophenotyping is an essential part of the diagnostic evaluation. Antibodies distinguish clusters of differentiation (CD) groups, but most

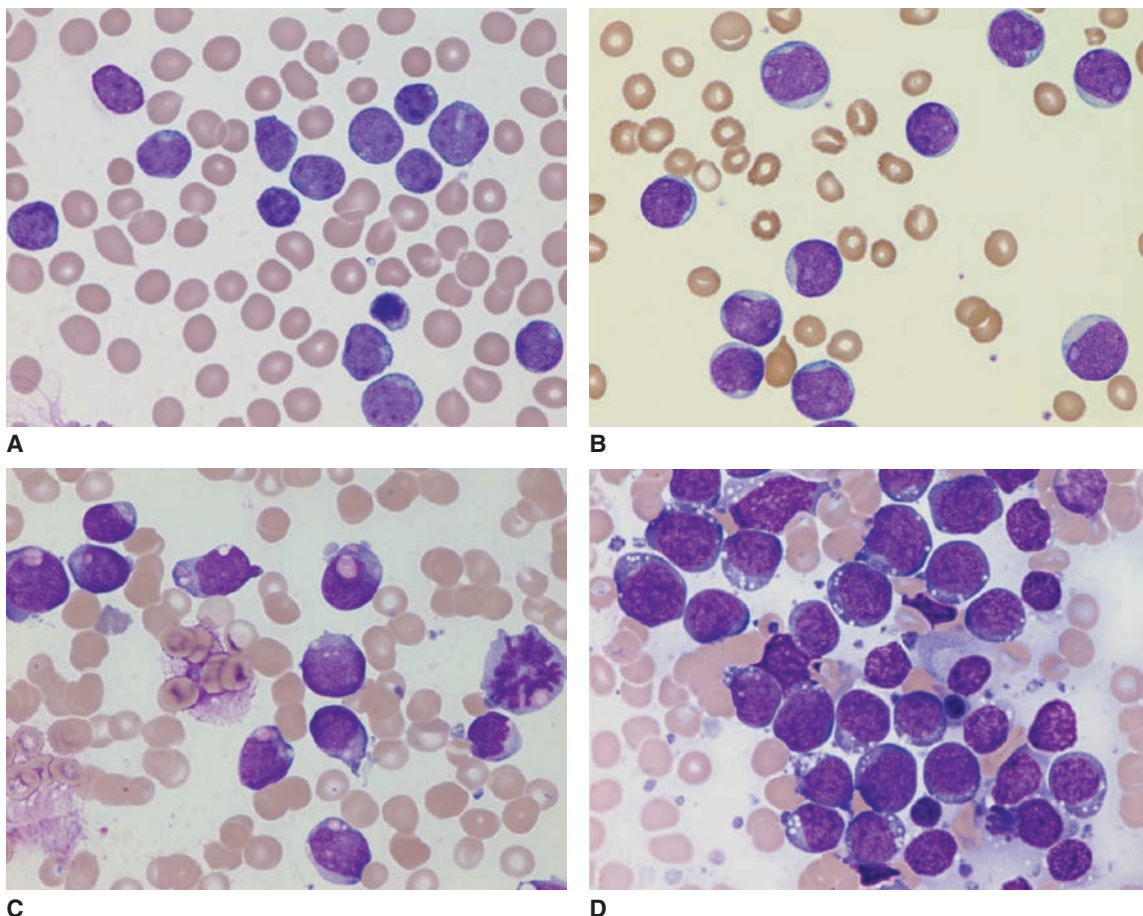


**Figure 2-4.** **A.** Typical lymphoblasts with scanty cytoplasm, regular nuclear shape, fine chromatin, and indistinct nucleoli. **B.** Acute lymphoblastic leukemia (ALL) with large blasts showing prominent nucleoli, moderate amounts of cytoplasm, and an admixture of smaller blasts. **C.** ALL with cytoplasmic granules. Fuchsia granules are present in the cytoplasm of many blasts. Such granules may lead to a misdiagnosis of acute myeloid leukemia; however, the granules are negative for myeloperoxidase and myeloid-pattern Sudan black B staining. **D.** B-cell ALL lymphoblasts. The blasts in this phenotype are characterized by intensely basophilic cytoplasm, regular cellular features, and cytoplasmic vacuolation. (Images **A** to **D**, Wright-Giemsa stain; original magnification  $\times 1000$ .)



leukocyte antigens lack specificity. Hence, a panel of antibodies is needed to establish the diagnosis and to distinguish among the different immunologic subclasses of leukemic cells. Typical panels include antibodies to at least one highly sensitive marker (CD19 for B-cell lineage, CD7 for T-cell lineage, and CD13 or CD33 for myeloid cells) and antibodies to a highly specific marker (cytoplasmic CD79a and CD22 for B-cell lineage, cytoplasmic CD3 for T-cell lineage, and cytoplasmic myeloperoxidase for myeloid cells).<sup>17</sup> Although ALL can be further subclassified according to the recognized steps of normal maturation within the B-cell lineage (pro-B, early pre-B, pre-B, transitional pre-B, and mature B cells) or T-cell lineage (pre-T, mid-, and late thymocyte) pathways, the only distinctions of therapeutic importance at present are those between T-cell, mature B, and other B-cell lineage (B-cell precursor type) immunophenotypes (Table 2-4).<sup>17</sup> A distinct subset of T-cell ALL that retains stem cell-like features, termed *early T-cell precursor ALL*, has been identified and associated with a dire prognosis with conventional chemotherapy.<sup>82</sup> Knowing the pattern of antigen expression at diagnosis is critically important for the detection of minimal residual disease by flow cytometry after treatment.<sup>83</sup>

Myeloid-associated antigens may be aberrantly expressed on otherwise typical lymphoblasts. Because of differences in monoclonal antibodies and immunophenotyping techniques, the frequencies of myeloid-associated antigen expression range from 5 to 30 percent in childhood cases and from 10 to 50 percent in adult cases.<sup>67,84</sup> The pattern of myeloid-associated antigen expression is correlated with certain genetic features of blast cells. CD15, CD33, and CD65 are expressed in ALL cases with a rearranged *MLL* gene, and CD13 and CD33 are expressed in cases with the *ETV6-RUNX1*.<sup>84</sup> There is a subset of cases that coexpress both lymphoid and myeloid markers but do not cluster with T-cell, B-cell precursor, or acute myeloid leukemia in gene-expression profiling. These cases may not respond to myeloid-directed therapy but attain remission with ALL-directed induction treatment.<sup>85,86</sup> The presence of myeloid-associated antigens lacks prognostic significance in

contemporary treatment programs but can be useful in immunologic monitoring of patients for minimal residual leukemia.<sup>67,83</sup>

### Genetic Classification

ALL arises from a lymphoid progenitor cell that has sustained multiple specific genetic alterations that lead to malignant transformation and proliferation. Thus, genetic classification of ALL yields more relevant biologic information than any other means. Approximately 75 percent of adult and childhood cases can be readily classified into prognostically or therapeutically relevant subgroups based on the modal chromosome number (or DNA content estimated by flow cytometry), specific chromosomal rearrangements, and molecular genetic changes.<sup>1,2,17-20,41,54-56,87-89</sup> Table 2-5 summarizes the prominent clinical and biologic features of cases with the most common genetic abnormalities.

Two ploidy groups (hyperdiploidy >50 chromosomes and hypodiploidy <44 chromosomes) have clinical relevance. Hyperdiploidy, which is seen in approximately 25 percent of childhood cases and in 6 to 7 percent of adult cases, is associated with a favorable prognosis that may reflect an increased cellular accumulation of methotrexate and its polyglutamates, an increased sensitivity to therapeutic antimetabolites, and a marked propensity of these cells to undergo apoptosis.<sup>90,91</sup> By contrast, hypodiploidy is associated with an exceptionally poor prognosis.<sup>88,89,92</sup> Flow cytometric determination of cellular DNA content is a useful adjunct to cytogenetic analysis because it is automated, rapid, and inexpensive, and its measurements are not affected by the mitotic index of the cell population; results can be obtained in almost all cases. Flow cytometric studies can sometimes identify a small but drug-resistant subpopulation of near-haploid cells that may be missed by standard cytogenetic analysis.

Reciprocal chromosomal translocations in cases of B-cell and T-cell ALL arise from errors in the normal recombination mechanisms that generate antigen receptor genes. Such rearrangements can fuse the promoter/enhancer element of the immunoglobulin heavy- or light-chain gene or

**TABLE 2-4.** Presenting Features of Acute Lymphoblastic Leukemia According to Immunologic Subtype

| Subtype                 | Typical Markers                                                                | Childhood (%) | Adult (%) | Associated Features                                                                                                                      |
|-------------------------|--------------------------------------------------------------------------------|---------------|-----------|------------------------------------------------------------------------------------------------------------------------------------------|
| B-cell precursor        | CD19+, CD22+, CD79a+, clg+/-, slgμ-, HLA-DR+                                   |               |           |                                                                                                                                          |
| Pro-B                   | CD10-                                                                          | 5             | 11        | Infants and adult age group, high leukocyte count, initial CNS leukemia, pseudodiploidy, <i>MLL</i> rearrangement, unfavorable prognosis |
| Early pre-B             | CD10+                                                                          | 63            | 52        | Favorable age group (1-9 years), low leukocyte count, hyperdiploidy (>50 chromosomes)                                                    |
| Pre-B                   | CD10+/-, clg+                                                                  | 16            | 9         | High leukocyte count, black race, pseudodiploidy                                                                                         |
| Mature B cell (Burkitt) | CD19+, CD22+, CD79a+, clg+, slg+ (kappa or lambda+)                            | 3             | 4         | Male predominance, initial CNS leukemia, abdominal masses, often renal involvement                                                       |
| T lineage               | CD7+, cCD3+                                                                    |               |           |                                                                                                                                          |
| T cell                  | CD2+, CD1+/-, CD4+/-, CD8+/-, HLA-DR-, TdT+/-                                  | 10            | 18        | Male predominance, hyperleukocytosis, extramedullary disease                                                                             |
| Pre-T                   | CD2-, CD1-, CD4-, CD8-, HLA-DR+/-, TdT+                                        | 1             | 6         | Male predominance, hyperleukocytosis, extramedullary disease, unfavorable prognosis                                                      |
| Early T-cell precursor  | CD1-, CD8-, CD5 <sup>weak</sup> , CD13+, CD33+, CD11b+, CD117+, CD65+, HLA-DR+ | 2             | ?         | Male predominance, age >10 years, poor prognosis                                                                                         |

cCD3, cytoplasmic CD3; clg, cytoplasmic immunoglobulin; slg, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase.

**TABLE 2-5.** Clinical and Biologic Features Associated with the Most Common Genetic Subtypes of Acute Lymphoblastic Leukemia

| Subtype                                         | Associated Features                                                                                                                                                                                                               | Estimated Event-Free Survival (%) |                  |
|-------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|------------------|
|                                                 |                                                                                                                                                                                                                                   | Children                          | Adults           |
| Hyperdiploidy (>50 chromosomes)                 | Predominant precursor B-cell phenotype; low leukocyte count; favorable age group (1–9 years) and prognosis in children                                                                                                            | 80–90 at 5 years                  | 30–50 at 5 years |
| Hypodiploidy (<45 chromosomes)                  | Predominant precursor B-cell phenotype; increased leukocyte count; poor prognosis                                                                                                                                                 | 30–40 at 3 years                  | 10–20 at 3 years |
| t(12;21)(p13;q22) [ETV6-RUNX1]                  | CD13+/-CD33+/- precursor B-cell phenotype; pseudodiploidy; age 1–9 years; favorable prognosis                                                                                                                                     | 90–95 at 5 years                  | Unknown          |
| t(1;19)(q23;p13.3) [TCF3-PBX1]                  | CD10+/-CD20-CD34- pre-B phenotype; pseudodiploidy; increased leukocyte count; black race; CNS leukemia; prognosis depends on treatment                                                                                            | 82–90 at 5 years                  | 20–40 at 3 years |
| t(9;22)(q34;q11.2) [BCR-ABL1]                   | Predominant precursor B-cell phenotype; older age; increased leukocyte count; myeloid antigens; improved early outcome with tyrosine kinase inhibitor treatment                                                                   | 80–90 at 3 years                  | ~60 at 1 year    |
| Ph-like ALL                                     | Characterized by a range of genomic alterations that activate a limited number of signaling pathways, all of which may be amenable to inhibition with tyrosine kinase inhibitors                                                  | 50 at 5 years                     | 25 at 5 years    |
| t(4;11)(q21;q23) with MLL-AF4 fusion            | CD10+/-CD15+/-CD33+/-CD65+/- precursor B-cell phenotype; infant and older adult age groups; hyperleukocytosis; CNS leukemia; poor outcome                                                                                         | 32–40 at 5 years                  | 10–20 at 3 years |
| t(8;14)(q24;q32.3)                              | Mature B-cell phenotype; L3 morphology; male predominance; bulky extramedullary disease; favorable prognosis with short-term intensive chemotherapy including high-dose methotrexate, cytarabine, and cyclophosphamide/ifosfamide | 75–85 at 5 years                  | 70–80 at 4 years |
| NOTCH1 mutations                                | T-cell phenotype; favorable prognosis                                                                                                                                                                                             | 90 at 5 years                     | 50 at 4 years    |
| HOX11 overexpression                            | CD10+ T-cell phenotype; favorable prognosis with chemotherapy alone                                                                                                                                                               | 90 at 5 years                     | 80 at 3 years    |
| Intrachromosomal amplification of chromosome 21 | Precursor B-cell phenotype; low white blood cell count; intensified treatment required to avert a poor prognosis                                                                                                                  | 30 at 5 years                     | ?                |

Data from Pui CH, Robison LL, Look AT: Acute lymphoblastic leukemia. *Lancet* 371:1030, 2008; Schultz KR, Bowman WP, Aledo A, et al: Improved early event free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: A Children's Oncology Group Study. *J Clin Oncol* 27:5715, 2009; Larson RA, Dodge RK, Burns CP, et al: A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. *Blood* 85:2025, 1995; Rizzieri DA, Johnson JL, Byrd JC, et al: Alliance for Clinical Trials in Oncology (ACTION): Improved efficacy using rituximab and brief duration, high intensity chemotherapy with filgrastim support for Burkitt or aggressive lymphomas: Cancer and Leukemia Group B study 10002. *Br J Haematol* 165(1):102-111, 2014; Roberts KG, Li Y, Payne-Turner D, et al: Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med* 371:1005, 2014.

the T-cell antigen receptor  $\beta/\gamma$  or  $\alpha/\delta$  gene to sites adjacent to a variety of transcription factor genes. More often, genetic rearrangements result from the fusion of two genes encoding different transcription factors. These chimeric genes encode active kinases and altered transcription factors that regulate genes involved in the differentiation, self-renewal, proliferation, and drug resistance of hematopoietic stem cells.

Specific cytogenetic findings are correlated with presenting clinical features, blast cell phenotypes, and clinical outcome (Table 2-5). However, there are now compelling reasons to focus on molecular genetic lesions. First, molecular analyses can identify important submicroscopic genetic alterations not visible by standard karyotyping, such as the *ETV6-RUNX1* fusion, intrachromosomal amplification of chromosome 21, deletions of tumor-suppressor genes, and mutations of protooncogenes.<sup>1,2,87,93-95</sup> Second, cases with clinically important genetic rearrangements can be missed because of technical errors (e.g., karyotyping residual normal metaphase cells rather than leukemic metaphase cells).

Hence, FISH and reverse transcriptase polymerase chain reaction (RT-PCR) assays are increasingly used. The application of microarray-based, genome-wide analysis of gene expression and DNA copy number, complemented by transcriptional profiling, resequencing and epigenetic approaches, and next-generation sequencing, have identified specific genetic alterations with biologic and therapeutic implications.

## ● DIFFERENTIAL DIAGNOSIS

The initial manifestations of ALL can mimic a variety of disorders. The acute onset of petechiae, ecchymoses, and bleeding can suggest idiopathic thrombocytopenic purpura. The latter disorder often is associated with a recent viral infection, large platelets in blood films, normal hemoglobin concentration, and absence of leukocyte abnormalities in blood or marrow. Patients with ALL, promyelocytic leukemia, or aplastic

anemia can present with pancytopenia and complications associated with marrow failure. However, in aplastic anemia, hepatosplenomegaly and lymphadenopathy are rare, and the skeletal pain associated with ALL is absent. The results of marrow aspiration or biopsy usually distinguish between these diseases, although the diagnosis can be difficult in a patient who has hypocellular marrow that is later replaced by lymphoblasts. In one study, transient pancytopenia preceded ALL in 2 percent of all pediatric cases.<sup>75</sup> During the preleukemic phase in these patients, polymerase chain reaction (PCR) analysis demonstrated monoclonality, suggesting inhibition of normal hematopoiesis by leukemia cells.<sup>96</sup> ALL should be considered in the differential diagnosis of patients with hyper-eosinophilia, which can be a presenting feature of leukemia or can precede its diagnosis by several months. Occasionally, hematogones in a regenerative marrow may mimic leukemic blast cells; flow cytometry with optimal combinations of antibodies may be required to distinguish them.<sup>97</sup>

Infectious mononucleosis and other viral infections, especially those associated with thrombocytopenia or hemolytic anemia, can be confused with leukemia. Detection of reactive lymphocytes or serologic evidence of Epstein-Barr virus infection helps establish the diagnosis. Patients with acute infectious lymphocytosis, pertussis, or paraptosis can have marked lymphocytosis. However, even when leukocyte counts are as high as  $50 \times 10^9/L$ , the affected cells are mature lymphocytes rather than lymphoblasts. Bone pain, arthralgia, and occasionally arthritis mimic juvenile rheumatoid arthritis, rheumatic fever, other collagen diseases, or osteomyelitis. The marrow should be examined if glucocorticoid treatment is planned for presumed rheumatoid diseases.

In children, ALL should be distinguished from small, round cell tumors involving the marrow, including neuroblastoma, rhabdomyosarcoma, and retinoblastoma. Generally, in patients with solid tumors, a primary lesion will be found by standard diagnostic studies. Disseminated tumor cells often present in characteristic aggregates, and immunophenotypic characteristics of lymphoblasts are absent.

## ● THERAPY

### SUPPORTIVE CARE

Optimal management of patients with ALL requires careful attention to supportive care, including immediate treatment or prevention of metabolic and infectious complications and rational use of blood products. Other important supportive care measures, such as use of indwelling catheters, amelioration of nausea and vomiting, pain control, and continuous psychosocial support for the patient and family, are essential.

### Metabolic Complications

Hyperuricemia and hyperphosphatemia with secondary hypocalcemia are frequently encountered at diagnosis, even before chemotherapy is initiated, especially in patients with B-cell or T-cell ALL or precursor B-cell leukemia with high leukemic cell burden.<sup>98</sup> Patients should be given intravenous fluids; allopurinol or rasburicase (recombinant urate oxidase) to treat hyperuricemia; and a phosphate binder, such as aluminum hydroxide, calcium carbonate (if the serum calcium concentration is low), lanthanum carbonate, or sevelamer to treat hyperphosphatemia. Allopurinol, a relatively inexpensive drug, is usually used if the uric acid is less than 7 mg/dL. Allergic skin reactions occur in approximately 10 percent, and allopurinol should be stopped as soon as the risk of hyperuricemia from the destruction of a large leukemic cell burden has passed. By inhibiting *de novo* purine synthesis in leukemic blast cells, allopurinol can reduce the peripheral blast cell count before chemotherapy.<sup>99</sup> Allopurinol can decrease both the anabolism and the catabolism of mercaptopurine by depleting intracellular phosphoribosyl pyrophosphate and by inhibiting xanthine oxidase. If mercaptopurine

and allopurinol are given together orally, the dosage of mercaptopurine must be reduced.

Rasburicase works very rapidly and is extremely effective, especially for very elevated uric acid levels ( $>7$  mg/dL), often with one infusion (a far smaller dose than the manufacturer recommends). Rasburicase breaks down uric acid to allantoin, a readily excreted metabolite that is five to 10 times more soluble than uric acid. Rasburicase is more effective than allopurinol, and it facilitates phosphorus excretion, partly because of rasburicase's potent uricolytic effect (which obviates the need to alkalinize urine) and partly because of improved renal function with its use.<sup>100</sup> However, rasburicase is contraindicated in patients with glucose-6-dehydrogenase deficiency because hydrogen peroxide, a by-product of uric acid breakdown, can cause methemoglobinemia or hemolytic anemia.

### Hyperleukocytosis

For patients with extreme leukocytosis (leukocyte count  $>400 \times 10^9/L$ ), either leukapheresis or exchange transfusion (in small children) can be used to reduce the burden of leukemic cells. In theory, either treatment should reduce the complications associated with leukostasis, but the short- and long-term benefits of the procedures are questionable.<sup>70</sup> Emergency cranial irradiation, once advocated by some leukemia therapists, probably has no role in the treatment of these patients. Preinduction therapy with low-dose glucocorticoids, with addition of vincristine and cyclophosphamide in cases of B-cell ALL, is a favored means of ameliorating hyperleukocytosis. This method, when used in conjunction with urate oxidase, has largely eliminated tumor lysis syndrome and the need for hemodialysis in patients with mature B-cell ALL.

### Infection Control

Infections are common in febrile patients with newly diagnosed ALL. Therefore, any patient presenting with fever, especially a patient with neutropenia, should be given broad-spectrum antibiotics until infection is excluded. Remission induction therapy can increase susceptibility to infection by exacerbating myelosuppression, immunosuppression, and mucosal breakdown. At least 50 percent of patients undergoing induction therapy experience infections. Special precautions should be taken to reduce the risk of infection during this critical phase of treatment, including protective contact isolation and air filtration; elimination of contact with people with infections; refraining from eating certain food products, such as raw cheese, uncooked vegetables, or unpeeled fruits; and use of antiseptic mouthwash or sitz baths, especially for patients with mucositis. Good hand-washing practices and the use of alcohol-based cleansers are important. Administration of granulocyte colony-stimulating factor can hasten recovery from neutropenia and reduce the complications of intensive chemotherapy but does not improve the EFS rate for children or adults.<sup>101,102</sup> One study suggested growth factor increased the risk of therapy-related acute myeloid leukemia in the context of epipodophyllotoxin-based therapy.<sup>103</sup> Intensified remission induction regimens, especially in combination with high-dose glucocorticoids, have resulted in an increased risk of disseminated fungal infection and death. Antifungal prophylaxis is commonly given.

All nonallergic patients with ALL are given trimethoprim-sulfamethoxazole, 2 to 3 days per week, as prophylactic therapy for *Pneumocystis carinii* (*Pneumocystis jiroveci*) pneumonia. Prophylaxis is started after 2 weeks of remission induction and continues for several months after completion of all chemotherapy. Alternative treatments for patients who cannot tolerate trimethoprim-sulfamethoxazole include aerosolized pentamidine, dapsone, and atovaquone.<sup>104</sup> Live-virus vaccines should not be administered during immunosuppressive therapy. Siblings and other children who have frequent contact with patients can receive routine immunizations, including inactivated poliomyelitis



vaccine. Susceptible patients exposed to varicella virus should receive zoster immunoglobulin within 96 hours of exposure together with acyclovir. Such treatment usually prevents or mitigates the clinical manifestations of varicella.

### **Hematologic Support**

ALL and its treatment lead to pancytopenia. Hemorrhagic manifestations are common but usually are limited to the skin and mucous membranes. Although rare, bleeding in the CNS, lungs, or gastrointestinal tract can be life threatening. Patients with extremely high leukocyte counts ( $>400 \times 10^9/L$ ) at diagnosis are more likely to develop such complications.<sup>70</sup> Coagulopathy attributable to disseminated intravascular coagulation, hepatic dysfunction, or chemotherapy is usually mild.<sup>76</sup> Patients receiving L-asparaginase and a glucocorticoid have a hypercoagulable state. Platelet transfusions should be given therapeutically for overt bleeding and may be used prophylactically when platelet counts are less than  $10 \times 10^9/L$ .<sup>105</sup> Anticoagulants and antiplatelet agents such as aspirin must be avoided. Children generally do not have active bleeding during remission induction therapy with prednisone, vincristine, and L-asparaginase, even when platelet counts are less than  $10 \times 10^9/L$ . A higher threshold for prophylactic platelet transfusions should be considered for active toddlers and patients with fever or infection. Transfusion of packed leukocyte-poor red cells is indicated in patients with anemia and marrow suppression but should be delayed until the leukocyte count is reduced in patients with extreme hyperleukocytosis. Transfusions should be given slowly in patients with profound but chronic anemia to prevent development of congestive heart failure. Granulocyte transfusions are rarely needed but should be considered for patients with absolute neutropenia and documented gram-negative septicemia or disseminated fungal infection that is responding poorly to antimicrobial treatment alone. All blood products should be irradiated to prevent transfusion-related graft-versus-host disease.

## **ANTILEUKEMIC THERAPY**

Because ALL is a heterogeneous disease with many distinct subtypes, there is no uniform approach to therapy. Increasingly, treatment is targeted to biologically distinct subgroups. The best results have been reported from experienced treatment centers using well-designed and rigorously applied protocols.<sup>106–109</sup> No consensus exists on the risk criteria and the terminology for defining prognostic subgroups. Usually, childhood ALL cases are divided into standard-risk, high- (intermediate- or average-) risk, and very-high-risk groups, although the U.S. Children's Oncology Group advocates four categories, including low risk, to accommodate patients with a very low risk of relapse. Adult cases are generally divided into two risk groups. Often infant and elderly ALL are considered special subgroups of ALL that require different treatment, primarily related to intolerance. One study showed improved outcome for infant ALL using a hybrid treatment protocol with elements to treat both ALL and acute myeloid leukemia and reducing dose intensity in the very young infants.<sup>110</sup> Few studies have been performed in those older than 60 years of age, and their management remains a therapeutic challenge.<sup>111,112</sup> Some successes have been achieved with the use of dose-reduced regimens and the addition of imatinib or dasatinib for patients with Philadelphia chromosome-positive ALL.<sup>113,114</sup> Because cure is not common in patients older than age 70 years, maintenance of a good quality of life is a major goal for this age group.

### **Mature B-Cell Acute Lymphoblastic Leukemia (Burkitt Type)**

The most effective contemporary treatment regimens for mature B-cell (Burkitt-type) ALL are drug combinations that include cyclophosphamide and/or ifosfamide given over a relatively short time (3 to 6 months).

The first major breakthrough in this disease was reported by French investigators, who achieved a 68 percent EFS rate in their LMB84 study featuring high-dose cyclophosphamide, high-dose methotrexate, vincristine, doxorubicin, and conventional doses of cytarabine. In the LMB89 study, the same group reported a cure rate of 87 percent, which was achieved by using increased doses of methotrexate (to  $8 \text{ g/m}^2$  per dose) and cytarabine ( $3 \text{ g/m}^2$  per dose) and by adding etoposide for patients with a large leukemia cell burden.<sup>115</sup> This excellent result has been confirmed in a randomized international study.<sup>116</sup> Successful treatments also have been developed by the Berlin-Frankfurt-Münster consortium, which uses a multiagent regimen that incorporates cyclophosphamide, high-dose methotrexate ( $1 \text{ g/m}^2$  per dose), etoposide, ifosfamide, doxorubicin, dexamethasone, and cytarabine ( $3 \text{ g/m}^2$  per dose).<sup>117</sup> These intensive pediatric protocols have been translated into adult treatment regimens that are completed in as little as 18 weeks.<sup>118–120</sup> Because of its demonstrated efficacy in B-cell lymphoma, rituximab (anti-CD20) has been incorporated in frontline clinical trials for adults with B-cell ALL.<sup>118,120,120a</sup> Maintenance or continuation therapy is not needed. The remission rate is very high, and most remissions are durable. B-cell ALL rarely, if ever, reoccurs after the first year.

Effective CNS therapy is an essential component of successful regimens for B-cell ALL and generally consists of methotrexate and cytarabine administered both systematically and intrathecally. Cranial irradiation does not appear to be necessary even for patients presenting with CNS leukemia.<sup>116,120</sup>

### **Precursor B-Cell and T-Cell Acute Lymphoblastic Leukemia**

Treatment for leukemias affecting the precursor B-cell and T-cell lineages consists of three standard phases: remission induction, intensification (consolidation), and prolonged continuation (maintenance) therapy.<sup>121</sup> CNS-directed therapy, which overlaps other treatments, is started early and is given for different lengths of time, depending on the patient's risk of relapse and the intensity of the primary systemic regimen.

**Remission Induction** The first goal of therapy is inducing a complete remission and restoring normal hematopoiesis. The induction regimen typically includes a glucocorticoid (prednisone, prednisolone, or dexamethasone), vincristine, and L-asparaginase for children or an anthracycline for adults.<sup>17,67,106–108</sup> Children with high- or very-high-risk ALL, and nearly all young adults with ALL, receive four or more drugs (daunorubicin, vincristine, glucocorticoid, and L-asparaginase) during remission induction in contemporary clinical trials. Improvements in chemotherapy and supportive care have resulted in complete remission rates of approximately 98 percent for children and 85 to 90 percent for adults. When a complete clinical remission is induced, patients have various degrees of residual leukemia.<sup>122</sup> Because the extent of residual disease is well correlated with long-term outcome,<sup>83,123–129</sup> the concept of a “molecular” or “immunologic” remission, defined as leukemia less than 0.01 percent of nucleated marrow cells,<sup>122</sup> is beginning to supplant the traditional perception of remission, which is based solely on microscopic criteria.<sup>129</sup> Prospective trials are needed to demonstrate that outcomes will improve when interventions to change therapy are based on measurements of residual disease.

Attempts have been made to intensify induction therapy based on the premise that more rapid and complete reduction of the leukemia cell burden forestalls the development of drug resistance. However, several studies have suggested intensive induction therapy is unnecessary for children with standard-risk ALL, provided patients receive postinduction intensification therapy.<sup>107</sup> Intensive induction can lead to increased early morbidity and mortality. More intensive induction regimens with additional cyclophosphamide, high-dose cytarabine, or high-dose anthracycline also have been tested in adults with ALL and have yielded no clear benefit, partly because of the low tolerance of adults to drug



toxicity.<sup>130,131</sup> However, in one study, the use of high-dose dexamethasone (10 mg/m<sup>2</sup> per day) instead of prednisone (60 mg/m<sup>2</sup> per day) during remission induction significantly improved treatment outcome for children with ALL, especially those with T-cell ALL and good prednisone response, despite a higher induction death rate.<sup>121,132</sup> Conceivably, intensified remission induction with other relatively nonmyelosuppressive drugs can also improve treatment outcome. Dexamethasone provided better control of systemic and CNS disease than did prednisone in two randomized studies of childhood ALL.<sup>133,134</sup>

The pharmacodynamics of asparaginase differ by formulation, and three forms are available: one derived from *Erwinia chrysanthemi*, another prepared from *Escherichia coli*, and a third made of a polyethylene glycol form of the *E. coli* product (pegaspargase).<sup>135,136</sup> In terms of leukemic control, the dose intensity and duration of asparaginase treatment (i.e., the amount of asparagine depletion) are far more important than the type of asparaginase used. The dosages of the three preparations are based on their half-lives. Pegaspargase, which has the longest half-life, usually is administered at 2500 IU/m<sup>2</sup> every other week for one to two doses in cases of newly diagnosed ALL. By contrast, the *Erwinia* preparation, which has the shortest half-life, is administered at 20,000 IU/m<sup>2</sup> three times per week for 6 to 12 doses. The doses of *E. coli* L-asparaginase range from 5000 to 10,000 IU/m<sup>2</sup>, administered two to three times per week for 6 to 12 doses. Because of lower immunogenicity, improved efficacy, and less-frequent administration, pegaspargase has replaced the native product as the first-line treatment for children and adults in the United States and is also increasingly used in other ALL trials around the world.<sup>137–140</sup> None of the various anthracyclines (daunorubicin, doxorubicin, idarubicin, and mitoxantrone) given to adults with ALL has proven superior to any other; daunorubicin is used most commonly.

**Intensification (Consolidation) Therapy** After normal hematopoiesis is restored, patients in remission become candidates for intensification therapy. Such treatment, administered shortly after remission induction, refers to readministration of the induction regimen or to high doses of multiple agents not used during the induction phase. Although there is no dispute on the importance of this treatment in childhood ALL, there is no consensus on the best regimen and duration of treatment. More commonly used regimens for childhood ALL include high-dose methotrexate with or without mercaptopurine, high-dose L-asparaginase given for an extended period, or a combination of dexamethasone, vincristine, L-asparaginase, and doxorubicin, followed by thioguanine, cytarabine, and cyclophosphamide.<sup>107,108,121,137,141,142</sup> This phase of therapy has improved outcomes, even for patients with low-risk ALL.<sup>143</sup> Patients with *ETV6-RUNX1* have an especially good outcome in clinical trials featuring intensive postremission treatment with glucocorticoids, vincristine, and asparaginase.<sup>144,145</sup> A very high dose of methotrexate (5 g/m<sup>2</sup>) appears to improve the treatment outcome of patients with T-cell ALL.<sup>141,146</sup> This finding is consistent with data indicating T-cell blasts accumulate methotrexate polyglutamates (active metabolites of the parent compound) less avidly than do B-cell precursors; consequently, higher serum levels of the drug are needed for an adequate therapeutic effect.<sup>147,148</sup> The conventional dose of methotrexate (1 g/m<sup>2</sup>) may be too low for many patients with B-cell precursor ALL. Among B-lineage ALL, blasts with either *ETV6-RUNX1* or *TCF3-PBX1* gene fusion accumulate significantly lower methotrexate polyglutamates compared to those with hyperdiploidy or other genetic abnormalities.<sup>149</sup> This finding suggested that patients with *ETV6-RUNX1* or *TCF3-PBX1* gene fusion would also benefit from a higher dose of methotrexate.

Based on pediatric studies, intensive consolidation therapy has become a standard in the treatment of adult ALL even though early studies failed to show the benefit of this phase of treatment. Various drugs have been used for intensification, including high-dose methotrexate,

high-dose cytarabine, cyclophosphamide, and asparaginase.<sup>67,102,150–153</sup> Increasingly, intensification treatment is risk adapted and subtype specific. In the German 06/93 study, high-dose methotrexate was used for patients with standard-risk B-cell precursor ALL, high-dose methotrexate and high-dose cytarabine for high-risk B-cell precursor ALL, and cyclophosphamide for T-cell ALL. The hyper-CVAD (cyclophosphamide, vincristine, Adriamycin, dexamethasone) regimen of the MD Anderson Cancer Center alternates the combination of cyclophosphamide, vincristine, doxorubicin (Adriamycin), and dexamethasone with high-dose methotrexate and high-dose cytarabine for four courses each. More recently, rituximab has been added for patients with CD20 expression on lymphoblasts.<sup>150,151</sup> In adults, methotrexate dose should probably be limited to 1.5 to 2.0 g/m<sup>2</sup> because higher doses may lead to excessive toxicities, delayed subsequent treatment, and reduced compliance. In a Cancer and Leukemia Group B study, a five-drug remission induction was followed by early and late intensification courses with eight drugs.<sup>67</sup> These studies and others suggested the benefit of early intensive consolidation therapy, especially in young adults. In adult T-cell ALL, benefit is derived from cyclophosphamide and cytarabine.<sup>67,102</sup> In other adult cases of standard-risk and high-risk ALL, the benefit is derived from high-dose cytarabine. Two German multicenter trials using high-dose cytarabine, mitoxantrone, and allogeneic hematopoietic stem cell transplantation showed markedly improved results in cases bearing the t(4;11), which generally confers an adverse prognosis.<sup>154</sup> Several ongoing trials are testing the efficacy of asparaginase during intensification in young adult ALL<sup>137</sup> because this drug clearly improves outcome in childhood ALL and is better tolerated during consolidation treatment than during remission induction.

Patients diagnosed with ALL between the ages of 16 and 39, often considered together as adolescents and young adults, are commonly treated by either adult or pediatric hematologists. Several retrospective comparative analyses have reported that adolescents and young adults with ALL treated on pediatric protocols have had superior EFS and overall survival rates when compared with similar patients enrolled on adult ALL trials. Preliminary data suggest that these patients have better outcomes when treated with pediatric-inspired regimens, and that excess toxicity is not observed.<sup>153,155–162</sup>

**Continuation Therapy** Although unnecessary for cure of mature B-cell leukemia, continuation therapy for 2 to 3 years is an integral part of pediatric and adult ALL regimens. Attempts to shorten the duration of treatment have led to inferior outcomes in both childhood and adult ALL,<sup>163</sup> although as many as two-thirds of childhood cases might be cured with only 12 months of treatment.<sup>164</sup> However, which subgroups of childhood ALL can be cured with abbreviated therapy is unclear. In a meta-analysis of 42 trials, a third year of continuation therapy reduced the likelihood of relapse during the third year, but no advantage to prolonging treatment beyond 3 years was observed.<sup>165</sup> Early studies demonstrated that the third year of continuation therapy benefits boys but not girls.<sup>166,167</sup> Hence, most studies discontinue all therapy for girls after 2 to 2.5 years of treatment. It is uncertain whether with improved contemporary treatment boys still require prolonged continuation treatment. Whether adults with ALL benefit from prolonged continuation therapy is also unclear. In most adult trials, continuation therapy is given for 2 years from diagnosis.

Methotrexate administered weekly and mercaptopurine administered daily constitute the usual continuation regimen for ALL. Accumulation of higher intracellular concentrations of the active metabolites of methotrexate and mercaptopurine and administration of this combination to the limits of tolerance (as indicated by low leukocyte counts) have been associated with improved clinical outcome.<sup>168,169</sup> Many investigators advocate that drug dosage be adjusted to maintain leukocyte counts below  $3 \times 10^9/L$  and neutrophil counts between 0.5 and  $1.5 \times 10^9/L$  to ensure adequate dose intensity during the continuation treatment in

childhood ALL.<sup>2</sup> In one study, the dose intensity of mercaptopurine was the most important pharmacologic factor influencing treatment outcome.<sup>170</sup> Mercaptopurine is most effective when it is given orally on a daily basis. However, overzealous use of mercaptopurine is counterproductive, as such use results in neutropenia and interruption of chemotherapy, reducing overall dose intensity. The effect of mercaptopurine is better when the drug is administered in the evening.<sup>171</sup> Mercaptopurine should not be given with milk or milk products containing xanthine oxidase, which can degrade the drug.<sup>172</sup> Antimetabolite treatment should not be withheld because of isolated increases of liver enzymes since such liver function abnormalities are tolerable and reversible.<sup>173</sup>

A few patients (one in 300) have an inherited homozygous deficiency of thiopurine S-methyltransferase, the enzyme that catalyzes the S-methylation (inactivation) of mercaptopurine. In these patients, standard doses of mercaptopurine have potentially fatal hematologic side effects. The drug should be given in much smaller doses (e.g., 10-fold reduction).<sup>174</sup> Approximately 10 percent of patients are heterozygous for the enzyme deficiency and have intermediate levels of thiopurine methyltransferase.<sup>175</sup> This subgroup can be treated safely with only moderate reductions in mercaptopurine dosage and appears to have better clinical outcomes than do patients with the homozygous wild-type phenotype. Importantly, patients with this enzyme deficiency are at risk for therapy-related myeloid leukemia and radiation-related brain tumors.<sup>176–178</sup> Identification of this autosomal codominant trait has been enabled by molecular diagnosis and led to increased emphasis on inherited differences in drug metabolism and disposition resulting from genetic polymorphisms in drug-metabolizing enzymes and in drug transporters, receptors, and targets.<sup>1,179,180</sup> Ultimately, therapy can be designed according to the genetic constitution of both the host and the host's leukemia cells.

Because thioguanine is more potent than mercaptopurine in model systems and leads to higher concentrations of thioguanine nucleotides in cells and cytotoxic concentrations in CSF,<sup>181</sup> randomized trials have been performed in children to compare the effectiveness of these two drugs.<sup>182</sup> Thioguanine, given at a daily dose of 40 mg/m<sup>2</sup> or more, produced superior antileukemic responses to mercaptopurine but was associated with profound thrombocytopenia, an increased risk of death, and an unacceptable rate of hepatic venoocclusive disease.<sup>182</sup> Consequently, mercaptopurine remains the drug of choice for ALL, although thioguanine is still used in short-term courses during the intensification phase of therapy.

Intermittent pulses of vincristine and a glucocorticoid improved the efficacy of antimetabolite-based continuation regimens and have been widely adopted for both childhood and adult ALL.<sup>165,183</sup> In older children and adults, prolonged glucocorticoid therapy may lead to increased risk of osteonecrosis.<sup>184</sup> Another integral component of many protocols is reinduction therapy introduced relatively soon after the first remission. This treatment, which relies on the same drugs used during the initial phase of induction therapy, has improved outcomes for children and adults with ALL.<sup>67,106–108</sup> A second reinduction phase during continuation treatment may further improve the outcome of patients with standard- or high-risk ALL.<sup>142,185</sup> The benefit of such a double-delayed intensification may result from either the increased dose intensity of other agents, such as asparaginase or anthracycline, or the timing or scheduling of the intensification regimen.

**Therapy of the Central Nervous System** The CNS is a common sanctuary for leukemic cells and requires prophylactic or presymptomatic therapy.<sup>78a</sup> In the 1970s, the cornerstone of ALL therapy was cranial irradiation (2400 cGy) plus methotrexate administered intrathecally after complete remission was induced. Concerns that cranial irradiation could cause second cancer, late neurocognitive deficits, and endocrinopathy stimulated efforts to replace cranial irradiation with early intensification by intrathecal and systemic chemotherapy. Two early

clinical trials tested the feasibility of complete omission of prophylactic cranial irradiation in the treatment of childhood ALL.<sup>186–188</sup> Although the cumulative risk of an isolated CNS relapse was relatively low (3 to 4 percent), the EFS rates in the two studies were only 68.4 percent and 60.7 percent.<sup>186–188</sup> In another study, prophylactic cranial irradiation appeared to improve outcome in T-cell ALL with leukocyte counts  $>100 \times 10^9/L$ .<sup>189</sup> Thus, until recently, virtually all childhood study groups continued to rely on prophylactic cranial irradiation for up to 20 percent of patients.<sup>80</sup> A radiation dose of 1200 cGy appeared to provide adequate protection against CNS relapse, even in high-risk patients (e.g., those with T-cell ALL and leukocyte counts  $>100 \times 10^9/L$ ).<sup>141</sup> A study at St. Jude Children's Research Hospital again tested the feasibility of total omission of prophylactic cranial irradiation in the context of risk-adapted intrathecal and systemic chemotherapy.<sup>190</sup> The 5-year survival rate for the 498 patients enrolled was 93.5 percent, and the cumulative risk of an isolated CNS relapse rate was only 2.7 percent, a promising result, suggesting that prophylactic cranial irradiation can be safely omitted in the context of the effective intrathecal and systemic chemotherapy. Another study by the Dutch Childhood Oncology Group also showed that prophylactic cranial irradiation can be safely omitted from children with ALL.<sup>191</sup> Preliminary data from additional trials also indicate that prophylactic cranial irradiation is not necessary.

Systemic treatment, including high-dose methotrexate, intensive asparaginase, and dexamethasone, as well as optimal intrathecal therapy, is important to control CNS leukemia.<sup>80,192</sup> Triple intrathecal therapy with methotrexate, cytarabine, and hydrocortisone is more effective than intrathecal methotrexate in preventing CNS relapse.<sup>193</sup> Because the presence of ALL blasts in the CSF, even from traumatic lumbar puncture, is associated with an increased risk of CNS relapse and poor EFS,<sup>80,81</sup> intrathecal therapy should be intensified in patients with this feature. With CNS prophylaxis and high-dose systemic therapy, most adults with ALL remain free of CNS disease. CNS disease at the time of leukemia relapse in adults occurs in approximately 10 percent of cases. The frequency of CNS recurrence is about the same whether CNS radiation therapy (12 to 24 Gy) is used or whether only intrathecal cytotoxic therapy is used. Systemic high-dose methotrexate and cytarabine add to the CNS therapy. The outcome after CNS relapse is poor. Survival after CNS relapse is usually less than 1 year in adults. Treatment of CNS relapse requires cranial irradiation, intrathecal chemotherapy (typically via an Ommaya shunt), plus reinduction and reconsolidation systemic therapy.

**Stem Cell Transplantation** Hematopoietic stem cell transplantation during first remission remains controversial.<sup>194</sup> In adult ALL, long-term disease-free survival rates range from 35 to 50 percent with chemotherapy alone and from 45 to 60 percent with allogeneic transplantation.<sup>195,196</sup> However, interpretation of these results is difficult because of the lack of true randomization. Even so, results from both adult and pediatric studies suggest allogeneic transplantation benefits some high-risk patients.<sup>194,196,197</sup> Because of their unfavorable prognosis, patients with the Philadelphia chromosome-positive ALL and those with a poor initial response to induction therapy have been recommended to undergo allogeneic stem cell transplantation during the first remission.<sup>194–196,198</sup> However, the advent of improved chemotherapy has diminished the survival advantage from transplantation in children with Philadelphia chromosome-positive ALL.<sup>199</sup> The use of a tyrosine kinase inhibitor has further improved the early treatment results,<sup>200</sup> casting doubt on the benefit from transplantation in first remission in childhood cases.<sup>201</sup> Allogeneic transplantation appears to improve the outcome of adults with the t(4;11),<sup>154</sup> but not that of children or infants with the same genotype.<sup>202</sup>

Allogeneic transplantation has not been compared to chemotherapy alone in a true randomized trial; thus, the results of comparative studies are biased by availability of appropriate donors and other factors.<sup>194,203–205</sup> The more potent antileukemia activity of allogeneic transplantation is

balanced against the considerable nonrelapse mortality and long-term consequences of graft-versus-host disease. A meta-analysis involving 3157 patients supported matched sibling donor allogeneic transplantation as the optimal postremission therapy for adults with ALL with a significant reduction in relapses and a significant increase in treatment-related mortality. Results may differ depending upon stem cell source, that is, related or unrelated donors or umbilical cord stem cells. A retrospective study of 421 adults who underwent allogeneic cord blood transplantation reported 2-year leukemia-free survival of 39 percent for patients in first complete remission and 31 percent for second remission. In multivariate analysis, factors associated with poor outcomes were age older than 35 years, myeloablative conditioning, and more advanced disease. Reduced intensity-conditioning allografting has yielded lower nonrelapse mortality and higher relapse rates than myeloablative conditioning, with no significant differences in leukemia-free survival.<sup>206,207</sup> The indications for allogeneic transplantation in first remission should be reevaluated as chemotherapy and transplantation continue to improve. Autologous transplantation failed to improve outcome in adult ALL overall, mainly because of a high rate of relapse.<sup>195</sup> Several small studies indicated that autologous stem cell transplantation is feasible and beneficial for adults with Philadelphia chromosome–positive ALL who achieve a molecular remission after combined chemotherapy and imatinib.<sup>208,209</sup>

**Targeted Therapies** The best example of targeted therapy is the use of the tyrosine kinase inhibitors (imatinib, dasatinib, nilotinib, or ponatinib) in Philadelphia chromosome–positive ALL.<sup>21,113,114,210,210a–210d</sup> Used as single agents or with a glucocorticoid, they can induce complete remission in older patients, for whom this subset of ALL is more common.<sup>114,210,211</sup> In combination with chemotherapy, they induce not only a higher complete remission rate but also a high rate of molecular remission in children and adults.<sup>200,201,210c,210d,212–216</sup> The duration of these remissions is uncertain, but some have been quite long after additional chemotherapy.<sup>210c</sup> Although the need for early transplantation in childhood cases is uncertain, this treatment modality is still a standard for adult cases.<sup>206,217</sup> The use of imatinib or dasatinib yields a higher proportion of adult cases suitable for transplantation. The outcome depends on minimal residual disease before and after transplantation.<sup>218</sup> In patients with residual disease after transplantation, rapid response to imatinib was associated with superior survival.<sup>219</sup> It is uncertain whether and when to discontinue imatinib or dasatinib after the patient is treated with chemotherapy or transplantation.

Surface expression of CD20 by leukemia cells is associated with an inferior outcome in adult,<sup>220</sup> but not childhood, ALL.<sup>221</sup> Chemotherapy trials incorporating rituximab, an anti-CD20 antibody, have yielded promising results in adults with CD20-positive B-cell precursor ALL.<sup>150,222</sup> In a randomized trial for adults under 60 years with CD20-positive, Ph-negative ALL, adding rituximab to chemotherapy improved EFS.<sup>222a</sup> Other monoclonal antibodies that bind CD22 and CD19 are in late-stage clinical development.<sup>223</sup> Blinatumomab is a bispecific monoclonal antibody that binds both CD3-positive T cells and CD19-positive ALL cells and has been approved for patients with relapsed or refractory B-cell precursor ALL.<sup>223a,223b</sup> Inotuzumab ozogamicin is an anti-CD22 antibody conjugated to calicheamicin that has yielded better outcomes in patients with relapsed or refractory ALL than standard chemotherapy.<sup>223c</sup> Nelarabine is an approved antimetabolite drug that has shown considerable activity in T-cell ALL, both alone and in combination with other chemotherapy.<sup>224–226</sup>

## COURSE AND PROGNOSIS

### RELAPSE

Relapse is defined as the reappearance of leukemia cells at any site in the body. Most relapses occur during treatment or within the first 2 years after its completion, although initial relapses have been observed 10 or more

years after diagnosis.<sup>227</sup> Molecular studies suggest that in some cases, especially those with the *ETV6-RUNX1* fusion, subsequent mutations of the residual preleukemic clone that were not eradicated during initial treatment account for the “late relapse.”<sup>228</sup> The marrow remains the most common site of relapse in ALL. Anemia, leukocytosis or leukopenia, thrombocytopenia, enlargement of the liver or spleen, bone pain, fever, or a sudden decrease in tolerance to continuation chemotherapy may signal the onset of marrow relapse. In contemporary treatment programs for childhood ALL, the rates of CNS and testicular relapse have decreased to 3 percent or less.<sup>190,192</sup> Leukemic relapse occasionally occurs at other extramedullary sites, including the eye, ear, ovary, uterus, bone, muscle, tonsil, kidney, mediastinum, pleura, and paranasal sinus.

For ALL patients who have received a modern, intensive multi-agent treatment regimen, with delayed intensification and prolonged continuation therapy, a relapse of disease portends very poor survival. Although some individuals can be rescued with additional chemotherapy alone, in general, only allogeneic hematopoietic stem cell transplantation offers a reasonable chance for cure and long-term survival. Thus, treatment for relapse is often considered as “a bridge to transplant.” Two new drugs, clofarabine and liposomal vincristine, were approved as single agents in large part based upon their ability to enable patients with relapsed ALL to proceed to a transplant.<sup>229,230</sup> Because the outcome of allogeneic transplantation is poor in the presence of overt ALL, reinduction therapy aims for rapid cytorreduction of lymphoblasts while efforts proceed concurrently to identify an HLA-matched donor. The optimal pretransplant conditioning regimen then depends largely on the age and medical condition of the patient.

Novel immunotherapeutic agents offer advantages over conventional chemotherapy for relapsed ALL. The bispecific T-cell engaging (BiTE) antibody blinatumomab achieves remissions as a single agent in 40 to 45 percent of patients with chemotherapy-refractory B-cell ALL.<sup>223a,223b</sup> Inotuzumab ozogamicin is an immunoconjugate monoclonal antibody that yields a high response rate in relapsed CD22-positive ALL.<sup>223c</sup> Chimeric antigen receptor (CAR)–engineered T cells can mount an autologous cellular immune attack against CD19-positive ALL cells that has resulted in long-term disease-free survival. This therapy can be complicated by a severe cytokine release syndrome in some cases.<sup>230a</sup>

Marrow relapse, with or without extramedullary involvement, portends a poor outcome for most patients.<sup>231,232</sup> Factors indicating an especially poor prognosis include relapse while on therapy or after a short initial remission, T-cell immunophenotype, the presence of the Philadelphia chromosome, and an isolated hematologic relapse.<sup>232–234</sup> Prolonged second remissions (>3 years) can be achieved with chemotherapy in as many as half of patients with late relapses (i.e., >6 months after cessation of therapy) but in only approximately 10 percent of those with early relapse. The persistence of minimal residual disease after reinduction treatment also portends a very poor prognosis.<sup>235</sup> In patients who develop hematologic relapse while on therapy or shortly thereafter, and in those with residual disease after remission induction for relapse, allogeneic hematopoietic stem cell transplantation is the treatment of choice.<sup>236</sup> Autologous transplantation as postinduction treatment offers no substantial advantage over chemotherapy.<sup>190,192,237</sup> For patients without histocompatible related donors, transplantation of stem cells from cord blood or marrow from matched unrelated donors is recommended.<sup>201,238–241</sup> For patients with ALL that has relapsed after allogeneic transplantation, a second transplant or donor T-lymphocyte infusion occasionally results in sustained remission.<sup>242</sup>

### Central Nervous System Relapse

Although extramedullary relapse is frequently an isolated clinical finding, many occurrences are associated with recurrent disease detectable in the marrow.<sup>78a</sup> CNS relapses are associated with a higher level of minimal residual disease in the marrow than testicular relapses.<sup>243</sup>



Submicroscopic marrow involvement at a level of  $10^{-4}$  or higher at the time of overt extramedullary relapse confers a very poor outcome.<sup>243</sup> Hence, patients with extramedullary relapse and detectable disease in marrow require intensive systemic treatment to prevent subsequent hematologic relapse. The efficacy of salvage therapy in children with an isolated CNS relapse depends partly on duration of first complete remission and partly on whether CNS irradiation was previously performed. The strategy of delaying cranial or craniospinal irradiation for 6 to 12 months to allow initial intensification with systemic chemotherapy has yielded long-term second EFS of 70 to 80 percent in children with isolated CNS relapse.<sup>244,245</sup> In one study, 12 months of intensive systemic chemotherapy and reduced-dose cranial irradiation (18 Gy) resulted in an excellent 4-year EFS rate among children with precursor B-cell ALL who had not received cranial irradiation during initial treatment and who had an initial remission duration of 18 months or more.<sup>246</sup> Notably, in this study, a favorable age group of 1 to 9.9 years plus a low presenting leukocyte count ( $<50 \times 10^9/L$ ) at diagnosis of ALL were independent favorable prognostic factors. For patients, especially those with T-cell ALL, in whom relapse develops during therapy and who had previously undergone cranial irradiation, the remission rate generally does not exceed 30 percent.<sup>244,245</sup> Adults with isolated CNS relapse fare much more poorly than children. However, the recommended treatment strategy remains the same: combining CNS-directed treatment with additional systemic chemotherapy.

### Testicular Relapse

One-third of patients with early testicular relapse and two-thirds of patients with late testicular recurrence became long-term survivors after salvage chemotherapy and testicular irradiation.<sup>232,247,248</sup> In one study, some patients with late isolated testicular relapses were successfully treated with chemotherapy that included very high-dose methotrexate, without the addition of radiation therapy.<sup>249</sup> The optimal treatment and prognosis for patients with relapse at unusual extramedullary sites are unclear. However, the same principles that apply to the clinical management of CNS or testicular relapse probably apply to this subgroup.

## TREATMENT SEQUELAE

Despite the increasing intensity of curative treatment for childhood ALL, judicious use of supportive care reduced the rate of early death from 8 percent in the early 1970s to less than 2 percent in the 1990s.<sup>16,17</sup> Currently, the induction mortality ranges between 2 and 11 percent in adult ALL, with increasing age associated with a higher death rate.<sup>66,67,150–153</sup> Most deaths are caused by bacterial or fungal infections. The death rate among older patients receiving remission induction therapy can be as high as 30 percent because of increased hematologic and nonhematologic toxicities (e.g., hepatotoxicity and cardiotoxicity).<sup>111,112</sup> This poor tolerance for chemotherapy and consequent reduction of dose intensity largely account for the poor clinical outcome in older patients.

Patients previously treated for ALL are best monitored in dedicated cancer survivorship clinics where both early and late complications can be identified and managed. Table 2–6 summarizes common side effects associated with antileukemia therapy. Hyperglycemia develops in 10 to 20 percent of children during induction therapy with prednisone, vincristine, and L-asparaginase but has no long-term consequence or prognostic implication; in some cases, short-term insulin treatment is required.<sup>250</sup> Adolescent and older adult age, obesity, a family history of diabetes mellitus, and Down syndrome are associated with increased susceptibility to hyperglycemia.<sup>250,251</sup> This induction regimen causes a hypercoagulable state leading to cerebral thrombosis, peripheral vein thrombosis, or both in as many as 5 percent of patients. Cerebral thrombosis should be distinguished from transient ischemic lesions (posterior reversible encephalopathy syndrome), which are associated with acute

hypertension and severe constipation.<sup>252</sup> These lesions are located at the watershed areas between the major cerebral arteries and generally are reversible. Cerebral thrombosis can be readily distinguished from transient ischemic lesions by MRI or computed tomography (Fig. 2–5). Occasionally, cerebral thrombosis may not be apparent by diagnostic imaging until a few days after the onset of symptoms and signs. Thrombotic complications (especially in leg veins or inferior vena cava) are also common in adults receiving asparaginase.<sup>136–140</sup>

Emphasis on the intensive use of vincristine, methotrexate, and glucocorticoids has led to an increased frequency of neurotoxicity<sup>252,253</sup> and of osteonecrosis.<sup>254</sup> Many long-term survivors of childhood ALL, especially those who received high cumulative doses of glucocorticoid, methotrexate, or cranial irradiation, have developed severe osteoporosis.<sup>254–257</sup> Early identification of bone lesions and the introduction of therapy to prevent fractures is recommended. Treatment with anthracyclines can produce severe cardiomyopathy, especially when anthracyclines are given in high cumulative and peak doses to children, and particularly young girls.<sup>258–260</sup> Prolonged infusion did not appear to reduce late cardiotoxicity compared to bolus administration.<sup>261</sup> The existence of a safe cumulative dose of anthracycline is controversial. Cardiac abnormalities are persistent and progressive years after anthracycline therapy.<sup>262</sup> In one study, dexrazoxane prevented or reduced anthracycline-induced cardiotoxicity without interfering with antileukemic activity.<sup>263,264</sup> In current pediatric trials, limited doses of anthracyclines are used, even for high-risk cases, to decrease the risk of subsequent cardiomyopathy. Most regimens for adult ALL restrict cumulative anthracycline dosage to levels associated with less than 5 percent risk of congestive heart failure.

Cranial irradiation has been implicated as the cause of numerous late sequelae in children, including second cancer, neurocognitive deficits, and endocrine abnormalities that can lead to obesity, short stature, precocious puberty, and osteoporosis.<sup>265–269</sup> In general, these complications are seen in girls more often than in boys and in young children more often than in older children or adults. Long-term followup studies of survivors of childhood ALL revealed a greater than 10 percent cumulative risk of second neoplasms after 30 years of observation and a higher-than-average mortality rate among patients who had received cranial irradiation.<sup>266,267</sup> Patients who had been irradiated also had a high unemployment rate and, among women, a low marital rate. Many children with profound deficiencies of growth hormone receive hormone replacement therapy, which permits attainment of acceptable final heights without an increased chance of relapse.<sup>270</sup>

The most devastating complication is the development of brain tumors and therapy-related myeloid leukemia.<sup>271,272</sup> Children who undergo cranial irradiation at age 6 years or younger are most susceptible to development of brain tumors.<sup>273</sup> Intensive use of antimetabolites before and during cranial irradiation also increases the risk of brain tumor.<sup>177</sup> The median latency period for high-grade brain tumors is 9 years; it is 20 years for low-grade tumors (e.g., meningioma).<sup>266,273</sup>

Therapy-related myeloid leukemia has been linked to intensive treatment with epipodophyllotoxins (teniposide and etoposide) as well as to underlying germline predisposition syndromes.<sup>273a</sup> The risk of disease depends on treatment schedule; concomitant use of other agents (e.g., L-asparaginase, alkylating agents, perhaps antimetabolites); and host pharmacogenetics.<sup>176,274</sup> The long-term survival rate for patients with this complication is very low, even when the patients undergo allogeneic stem cell transplantation. No evidence indicates an increased incidence of cancer or birth defects among the offspring of adult survivors of childhood ALL.<sup>275,276</sup>

## PROGNOSTIC FACTORS

The cornerstone of the modern therapeutic approach to childhood ALL has been careful assessment of the risk of relapse so that only high-risk



**TABLE 2-6.** Side Effects Associated with Antileukemic Therapy

| Treatment                                            | Acute Complications                                                                                                                                                                                              | Late Complications                                                                                                                                                                                                        |
|------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Prednisone (or prednisolone)                         | Hyperglycemia, hypertension, changes in mood or behavior, acne, increased appetite, weight gain, peptic ulcer, hepatomegaly, myopathy                                                                            | Avascular necrosis of bone, osteopenia, growth retardation                                                                                                                                                                |
| Dexamethasone                                        | Same as prednisone, except for increased changes in mood or behavior and myopathy, but less salt retention                                                                                                       | Same as prednisone                                                                                                                                                                                                        |
| Vincristine                                          | Peripheral neuropathy, constipation, chemical cellulitis, jaw pain, seizures, hair loss                                                                                                                          |                                                                                                                                                                                                                           |
| Daunorubicin, idarubicin, doxorubicin, or epirubicin | Nausea and vomiting, hair loss, mucositis, marrow suppression, chemical cellulitis, increased skin pigmentation                                                                                                  | Cardiomyopathy (after high cumulative dose)                                                                                                                                                                               |
| L-Asparaginase                                       | Nausea and vomiting, allergic reactions (manifested as rashes, bronchospasm, severe pain at intramuscular injection site), hyperglycemia, pancreatitis, liver dysfunction, large vein thrombosis, encephalopathy | None                                                                                                                                                                                                                      |
| Mercaptopurine                                       | Nausea and vomiting, mucositis, marrow suppression, solar dermatitis, liver dysfunction; increased hematologic toxicity in persons lacking thiopurine methyltransferase                                          | Osteoporosis (long-term use), acute myeloid leukemia in persons with thiopurine methyltransferase deficiency                                                                                                              |
| Methotrexate                                         | Nausea and vomiting, liver dysfunction, marrow suppression, mucositis (resulting from high-dose treatment), solar dermatitis                                                                                     | Leukoencephalopathy, osteopenia (resulting from long-term use)                                                                                                                                                            |
| Etoposide, teniposide                                | Nausea and vomiting, hair loss, mucositis, marrow suppression, allergic reactions (bronchospasm, urticaria, angioedema, hypotension)                                                                             | Acute myeloid leukemia                                                                                                                                                                                                    |
| Cytarabine                                           | Nausea and vomiting, fever, skin rashes, mucositis, marrow suppression, liver dysfunction, conjunctivitis (from high-dose treatment)                                                                             | Decreased fertility (with high cumulative dose)                                                                                                                                                                           |
| Cyclophosphamide                                     | Nausea and vomiting, hemorrhagic cystitis, marrow suppression, syndrome of inappropriate secretion of antidiuretic hormone, hair loss                                                                            | Bladder cancer or acute myeloid leukemia (rare), decreased fertility (with high cumulative dose)                                                                                                                          |
| Rituximab                                            | Infusion reactions, mucocutaneous reactions, lymphopenia, pulmonary infiltrates                                                                                                                                  | Reactivation of virus infections (hepatitis B), progressive multifocal leukoencephalopathy from JC virus infection                                                                                                        |
| Intrathecal methotrexate                             | Headache, fever, seizure, marrow suppression, mucositis (in patients with renal dysfunction)                                                                                                                     | Encephalopathy or myelopathy (with high cumulative dose)                                                                                                                                                                  |
| Brain irradiation                                    | Hair loss, postirradiation somnolence syndrome (6–10 weeks after treatment)                                                                                                                                      | Seizure, mineralizing microangiopathy, growth hormone deficiency, thyroid dysfunction, obesity, osteopenia, brain tumors, basal cell carcinoma, parotid gland carcinoma, hair loss, cataract (rare), dental abnormalities |

or very high-risk patients are treated with intensive therapy.<sup>277,278</sup> Less toxic treatments (usually antimetabolites) are reserved for low-risk or standard-risk patients. By contrast, almost all adult patients are candidates for intensive therapy. Of the many variables that influence prognosis, treatment is the most important. Some of the factors that emerged as useful prognostic indicators in the past have disappeared as treatment has improved; others have shown predictive strength in one or several trials, but not in others. For example, T-cell ALL, once associated with a poor prognosis, now has long-term response rates of 70 to 85 percent in children<sup>2,17,190</sup> and 50 to 60 percent in adults<sup>66,67,279</sup> as a result of effective intensive chemotherapy. Outcomes for mature B-cell ALL, also a poor prognostic subset in the past, have improved in both children and adults, and 80 percent or more are cured with short but intensive chemotherapy treatments.<sup>280</sup>

Age and leukocyte count continue to be used for risk classification in almost every pediatric clinical trial involving precursor B-cell ALL. In a workshop sponsored by the National Cancer Institute, participants agreed on a presenting age of between 1 and 9 years and a leukocyte count of less than  $50 \times 10^9/L$  as the minimum criteria for low-risk ALL. These criteria apply only to precursor B-cell ALL and not to T-cell ALL. Among adults, the outcome of therapy worsens with increasing age and leukocyte count. Age younger than 35 years and leukocyte count less than  $30 \times 10^9/L$  are considered favorable prognostic indicators (Table 2-7).<sup>67,150,152</sup> In general, age younger than 60 years is considered a practical guide for selecting candidates who might benefit from intensive therapy, including allogeneic transplantation. Any decision to begin aggressive treatment in patients older than age 60 years must be weighed against the risk of increased morbidity and mortality.<sup>111,112</sup>

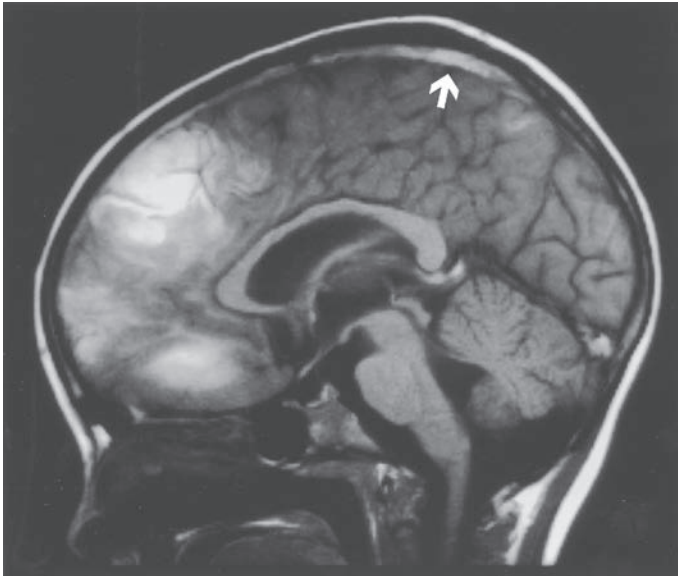


**Figure 2-5.** T1-weighted magnetic resonance image without contrast demonstrates a clot in the superior sagittal sinus (arrow) and several frontal lobe hematomas.

Male sex has long been recognized as an adverse prognostic factor in childhood ALL but has less influence in adult ALL. Its prognostic significance was abolished in a number of childhood studies in which overall outcome was improved. Black race conferred a poor outcome in the national clinical trials,<sup>281,282</sup> but in a single-institution study with equal access to effective treatment regimens, race had no prognostic significance.<sup>283</sup>

Primary genetic abnormalities have important prognostic significance. Hyperdiploidy (>50 chromosomes) and *ETV6-RUNX1* fusion—seen primarily in children ages 1 to 9 years but very rarely in adults—are associated with a favorable prognosis.<sup>2,17</sup> *MLL* rearrangements, which occur in 70 to 80 percent of infants younger than age 1 year and in 10 percent of adults, and Philadelphia chromosome with *BCR-ABL1*

**TABLE 2-7.** Adverse Prognostic Factors in Adult Acute Lymphoblastic Leukemia

| Factors                             | Precursor B Cell                                    | Precursor T Cell                                    |
|-------------------------------------|-----------------------------------------------------|-----------------------------------------------------|
| Age (years)*                        | >35                                                 | >35                                                 |
| Leukocyte count ( $\times 10^9/L$ ) | >30                                                 | >100                                                |
| Immunophenotype                     | Pro-B (CD10–)                                       | Pre-T                                               |
| Genetics                            | t(9;22) [ <i>BCR-ABL1</i> ]                         | <i>HOX11L2</i> expression?                          |
|                                     | t(4;11) [ <i>MLL-AF4</i> ]                          | <i>ERG</i> expression?                              |
|                                     | Hypodiploidy                                        |                                                     |
|                                     | Ph-like genotype                                    |                                                     |
| Treatment response                  | Delayed remission (>4 weeks)                        | Delayed remission (>4 weeks)                        |
|                                     | Minimal residual disease $>10^{-4}$ after induction | Minimal residual disease $>10^{-4}$ after induction |

\*Continuous factor with increasing age associated with progressively worse outcome.

**TABLE 2-8.** Risk Classification System Used in St. Jude Total Therapy Study XVI

| Risk Group | Feature                                                                                                                                                                                                                                                                                                                                                                                        |
|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Standard   | Precursor B-cell phenotype in patients ages 1–9 years with a presenting leukocyte count $<50 \times 10^9/L$ , <i>ETV6-RUNX1</i> fusion, or hyperdiploidy (>50 chromosomes or DNA index $>1.16$ )<br><br>Must not have CNS3 status, testicular leukemia, t(9;22), t(1;19), rearranged <i>MLL</i> gene, hypodiploidy, or $\geq 0.01\%$ leukemia cells in marrow after 6-week remission induction |
| High       | T-cell acute lymphoblastic leukemia (ALL) and all cases of B-cell precursor ALL that do not meet the criteria for standard or very high-risk ALL                                                                                                                                                                                                                                               |
| Very high  | Early T-cell precursor, initial induction failure, or $\geq 1\%$ leukemic cells in marrow after 6-week remission induction                                                                                                                                                                                                                                                                     |

fusion, which is found in 3 percent of children but in 25 to 30 percent of adult patients, historically confer a poor outcome.<sup>1,2,17,199</sup> Interestingly, there is a marked influence of age on the prognosis of genetic subtypes of ALL. For example, Philadelphia chromosome–positive ALL is associated with a poor outcome in adolescents but a relatively favorable outcome in children ages 1 to 9 years old who have a low leukocyte count at presentation.<sup>198</sup> Once associated with a dismal outcome, the early treatment outcome of Philadelphia chromosome–positive ALL in both children and adults has improved substantially with the advent of *BCR/ABL1* tyrosine kinase inhibitors.<sup>113,114,200,201,210–216</sup> Among patients with *MLL*-rearranged ALL, infants younger than age 1 year fare considerably worse than older children.<sup>202</sup> The basis of these differences may be related to some combination of secondary genetic events, the developmental stage of the target cell undergoing malignant transformation, and the pharmacogenetics or pharmacokinetic features of the patient. In T-cell ALL, *NOTCH1* or *FBXW7* mutation identifies a subgroup of childhood or adult cases with a favorable outcome.<sup>284–286</sup>

A useful adjunct in risk assessment is the response to early treatment, as measured by the rate of clearance of leukemia cells from the blood or marrow with the use of flow cytometric detection of aberrant immunophenotype or analysis by PCR of clonal antigen receptor gene rearrangements.<sup>83,123–129,190,287,288,288a</sup> This measure reflects the drug sensitivity or resistance of leukemia cells and the pharmacodynamics of the drugs, which is affected by the pharmacogenetics of the host. Current techniques allow measurement of minimal residual disease in all patients, and it has become the most important prognostic factor (Table 2-8). The expectation is that alteration of treatment intensity according to the level of minimal residual disease at early time points will improve the long-term outcome of patients with ALL. The level of measurable residual leukemia is also a strong predictor of treatment outcome before allogeneic stem cell transplantation for relapsed leukemia.<sup>235,289,290</sup> Pediatric trials have shown better outcomes when treatment decisions have been based in part on measurable levels of residual disease. However, similar benefit remains to be shown convincingly in adults.

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## CHAPTER 3

# CHRONIC LYMPHOCYTIC LEUKEMIA

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### SUMMARY

Chronic lymphocytic leukemia is a malignancy of mature B cells characterized by progressive lymphocytosis, lymphadenopathy, splenomegaly, and cytopenias. The progressive accumulation of leukemic B cells is a consequence of defective apoptosis and survival signals derived from the microenvironment. Progressive disease results in dysregulation of the cellular and humoral components of the effector immune system, with a resultant increase in the incidence of infectious complications, which constitutes the leading cause of morbidity and mortality in this disease. Significant therapeutic advances have been realized in recent years, especially with the development of well-tolerated targeted antibodies and kinase inhibitors. Although not curative, these therapies have resulted in significant improvements in patient outcomes with substantial increases in progression-free and overall survival intervals. Multiple novel agents are also in development with the potential to alter the treatment paradigms for this disease and ultimately to effect a cure.

### DEFINITION AND EPIDEMIOLOGY

Chronic lymphocytic leukemia (CLL) is one of the most common leukemias in the Western Hemisphere. CLL is a malignant lymphoid neoplasm that is characterized by the accumulation of a population of small mature B cells. The diagnosis of CLL requires the presence of at least 5000 circulating B cells/ $\mu\text{L}$ , with clonality demonstrated by flow cytometry according to International Workshop on Chronic Lymphocytic Leukemia (IWCLL) criteria.<sup>1</sup> Over the last two centuries, significant strides have been made in the understanding of the disease pathophysiology, clinical features, and complications arising from CLL. CLL was initially described by Virchow in the 1840s concerning patients with lymph node enlargement and leukocytosis. Subsequent studies revealed the involvement of the spleen and marrow and led to the introduction of the term *lymphosarcoma*. Ensuing natural history studies established the malignant and clonal nature of the disease and categorized patients based on clinical presentation. Surveillance, Epidemiology, and End Results (SEER) program data from 2013 estimated the prevalence of CLL in the United States at 126,553 patients, of whom 72,569 were males. The American Cancer Society estimated 15,720 new cases of CLL in 2014, with a median age of diagnosis of 72 years. This cancer is more common in men,<sup>2</sup> uncommon in patients younger than the age of 40 years, and extremely rare in children. The risk also increases progressively with age<sup>3</sup> and decreases with increasing parity in women.<sup>4</sup> It is also relatively uncommon in Asians,<sup>5</sup> even in Asian immigrants to the Western Hemisphere,<sup>6</sup> suggesting a possibility of a genetic predisposition. In the last few years, there has been tremendous growth in the understanding of the disease biology, which has resulted in the development of numerous new therapeutic options with resultant transformation in the management of this illness. Despite the significant improvement in the prognosis of this disease, cure currently remains elusive.

**Acronyms and Abbreviations:** ABC, activated B cell; ABVD, Adriamycin, bleomycin, vinblastine, and dacarbazine; ADCC, antibody-dependent cell-mediated cytotoxicity; ADP, adenosine diphosphate; AIHA, autoimmune hemolytic anemia; ALL, acute lymphoblastic lymphoma; ARLTS1, ADP-ribosylation factor-like tumor-suppressor gene 1; ATM, ataxia-telangiectasia mutated; BAK, Bcl-2 homologous antagonist/killer; BCL-2, B-cell lymphoma-2; BCR, B-cell receptor; BiTE, bispecific T-cell engaging; BR, bendamustine and rituximab; BTK, Bruton tyrosine kinase; CALGB, Cancer and Leukemia Group B; CAP, cyclophosphamide, doxorubicin, and prednisone; CAR-T, chimeric antigen receptor T cell; CD, cluster of differentiation; CDC, complement-dependent cytotoxicity; CDK, cyclin-dependent kinase; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CIRIS, cumulative illness rating scale; CLL, chronic lymphocytic leukemia; CLL-IPI, CLL–International Prognostic Index; CMP, cyclophosphamide, melphalan, and prednisone; CMV, cytomegalovirus; CR, complete response; CRi, complete response with incomplete count recovery; CT, computed tomography; CVP, cyclophosphamide, vincristine, and prednisone; CXCR4, C-X-C chemokine receptor type 4; DAPK, death-associated protein kinase; del, deletion; ERK1, extracellular signal-regulated kinase 1; FC, fludarabine and cyclophosphamide; FCR, fludarabine, cyclophosphamide, and rituximab; FDG-PET, fluorodeoxyglucose positron emission tomography; FISH, fluorescent *in situ* hybridization; FR, fludarabine and rituximab; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GVL, graft-versus-leukemia; HCL, hairy cell leukemia; HCT, hematopoietic cell transplantation; HLA, human leukocyte antigen; Ig, immunoglobulin; IGH, immunoglobulin heavy chain; IGHV, immunoglobulin heavy-chain variable region; IL, interleukin; ITK, IL-2–inducible T-cell kinase; ITP, immune thrombocytopenia; IVIG, intravenous immunoglobulins; IWCLL, International Workshop on Chronic Lymphocytic Leukemia; KLHL6, Kelch-like protein-6; LDH, lactate

dehydrogenase; LYN, Lck/Yes novel; MBL, monoclonal B-cell lymphocytosis; MCL-1, myeloid cell leukemia-1; MHC, major histocompatibility complex; miRNA, microRNA; MMP, matrix metalloproteinase; MMR, measles, mumps, and rubella; MRD, minimal residual disease; MYD88, myeloid differentiation primary response gene 88; NAD, nicotinic acid adenine; NCCN, National Comprehensive Cancer Network; NFAT, nuclear factor of activated T cells; NF- $\kappa$ B, nuclear factor kappa B; NK, natural killer; NOTCH1, Notch homologue 1, translocation-associated; NRM, nonrelapse mortality; OFAR, oxaliplatin, fludarabine, and rituximab; ORR, overall response rate; OS, overall survival; PCR, pentostatin, cyclophosphamide, and rituximab; PCV-13, pneumococcal 13-valent conjugate vaccine; PD-1, programmed cell death-protein 1; PFS, progression-free survival; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PLC $\gamma$ , phospholipase C-gamma-2; PLL, prolymphocytic leukemia; PR, partial response; PR+L, partial response with lymphocytosis; PRCA, pure red cell aplasia; RB, retinoblastoma; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-EPOCH, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; R-hyperCVXD, fractionated cyclophosphamide, vincristine, liposomal daunorubicin, and dexamethasone; RS, Richter syndrome; SDF-1, stromal cell–derived factor-1; SF3B1, splicing factor 3B subunit 1; SLL, small lymphocytic B-cell lymphoma; SNP, single nucleotide polymorphism; STAT3, signal transducer and activator of transcription 3; SUV, standardized uptake value; SYK, spleen tyrosine kinase; TCL1, T-cell leukemia/lymphoma protein 1A; TGF- $\beta$ , transforming growth factor- $\beta$ ; T-LGL, T-cell large granular lymphoma; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; TP53, tumor protein p53; T-PLL, T-cell prolymphocytic lymphoma; TRAP, tartrate-resistant acid phosphatase; TRM, transplant-related mortality; VCAM, vascular cell adhesion molecule; XIAP, X-linked inhibitor of apoptosis protein; XPO1, gene encoding exportin-1; ZAP-70, zeta-chain–associated protein kinase of 70 kDa.

## ● ENVIRONMENTAL FACTORS

Multiple studies have been conducted in an attempt to identify environmental factors that predispose people to the development of CLL. These studies have consistently identified a family history of hematologic malignancies as a strong predictive factor for the development of CLL.<sup>7,8</sup> In the reported International Lymphoma Epidemiology Consortium (InterLymph) Non-Hodgkin Lymphoma Subtypes Project,<sup>9</sup> detailed correlative studies were performed on a large cohort of white patients with CLL as compared to normal controls. The InterLymph study identified multiple factors that were associated with the presence of CLL, including (1) family history of a first-degree relative with hematologic malignancy, including lymphomas, leukemias, and myeloma; (2) a history of working or living on a farm; (3) occupation as hairdresser; and (4) a history of hepatitis C infection. Factors that were found to be protective included a history of allergies, blood transfusions, sun exposure, and smoking. CLL is also recognized as a service-connected illness among Vietnam War veterans who were exposed to Agent Orange.<sup>10</sup> Limited data suggest a possible risk of CLL in individuals chronically exposed to electromagnetic fields.<sup>11,12</sup> Radiation exposure, however, has not been shown to correlate with the development of CLL, as revealed by population-based studies of survivors of the Hiroshima atomic bomb and studies of nuclear reactor workers.<sup>13,14</sup> A smaller study conducted on survivors of the Chernobyl nuclear power plant accident did, however, suggest a slightly higher incidence of CLL in these people.<sup>15</sup>

## ● HEREDITARY FACTORS

CLL has a strong familial predisposition, with up to 10 percent of patients with a first- or second-degree relative with CLL and an even higher percentage when also considering individuals with monoclonal B-cell lymphocytosis.<sup>16,17</sup> Risk of acquiring CLL is also potentially increased in patients with first-degree relatives with other indolent non-Hodgkin lymphomas, including lymphoplasmacytic lymphomas.<sup>18</sup> Death-associated protein kinase (DAPK) and CD57 (LEU7) germline mutations have been linked to familial predisposition in a single CLL family.<sup>19</sup> Association studies have identified multiple putative genes, polymorphisms, and genetic factors, including CD5,<sup>20</sup> CD38,<sup>21</sup> tumor necrosis factor (TNF)  $\alpha$ ,<sup>22</sup> and human leukocyte antigen (HLA) haplotypes,<sup>23</sup> among others,<sup>24</sup> but definite mechanistic studies demonstrating clear contribution to pathogenesis are lacking.

## ● DISEASE BIOLOGY

CLL has varied presentations and complex biology that is the focus of ongoing studies of particular relevance to the practicing oncologist. CLL cells are derived from the B-lymphocyte lineage, as demonstrated by their expression of the pan-B-cell surface markers, including CD19, and a weaker expression of CD20.<sup>25,26</sup> Furthermore, CLL B cells express the memory B-cell marker CD27<sup>27</sup> and exhibit similar microarray profiles, suggesting a potential relationship to the normal memory B cell.<sup>28,29</sup> Most CLL B cells also express  $\kappa$  and  $\lambda$  immunoglobulin (Ig) light chains on their surface, along with M and D immunoglobulin heavy chains (IGHs).<sup>30,31</sup> These Igs are often reactive toward self-antigens and polyreactive<sup>32,33</sup> and may play a role in the survival and expansion of the leukemia cell clone.

CLL is characterized by gradual accumulation of leukemic cells, primarily from defective apoptosis that is partly contributed by microenvironment interaction. Overexpression of multiple antiapoptotic proteins like BCL-2 (B-cell lymphoma-2), MCL-1 (myeloid cell leukemia-1),<sup>34,35</sup> BAK (Bcl-2 homologous antagonist/killer), and XIAP

(X-linked inhibitor of apoptosis protein) along with transcription factors like NF- $\kappa$ B (nuclear factor kappa B), NFAT (nuclear factor of activated T cells), and STAT3 (signal transducer and activator of transcription 3) have been clearly demonstrated in CLL.<sup>36</sup> Additional survival signals are provided by the microenvironment and include cellular factors like nurse-like cells<sup>37</sup> and various chemokines like CXCR4 (C-X-C chemokine receptor type 4) and SDF-1 (stromal cell-derived factor-1).<sup>38</sup> A combination of these factors results in providing the CLL cells with a survival and proliferative advantage. CLL B cells exhibit differential proliferation in the various disease compartments, including the blood, spleen, and marrow.<sup>39,40</sup> CLL B cells isolated from the blood of patients lack proliferative potential *in vitro* and are restricted to the resting phase of the cell cycle.<sup>41,42</sup> These cells also undergo spontaneous apoptosis in routine culture conditions. Their survival can be extended when these cells are cultured on stromal cells or nurse-like cells that are generally found in the secondary lymphoid organs.<sup>43,44</sup> These secondary lymphoid organs are generally diffusely infiltrated by the B cells and are potentially the sites of cell division and proliferation.<sup>45</sup> *In vivo*, the leukemic cell clones increase by 0.1 to 1 percent per day despite a stable blood lymphocyte count as assessed by elegant heavy water studies.<sup>46</sup>

## ● IMMUNE DYSREGULATION

CLL is characterized by progressive immune dysregulation in both the cellular and the humoral compartments.<sup>47</sup> Progression of CLL is associated with an early increase in the absolute number of circulating T cells and specifically an increase in the immunosuppressive T-regulatory cells.<sup>48,49</sup> Functional studies of T cells from patients with CLL have also shown the T cells to be anergic and with impaired proliferative potential, but with a retained capacity to produce cytokines.<sup>50</sup> Functional defects have also been observed in granulocytes.<sup>51</sup> The leukemic B cells are responsible for initiating and propagating the immune dysregulation observed in the disease by producing immunosuppressive cytokines like transforming growth factor- $\beta$  (TGF- $\beta$ ) or by downregulating critical surface molecules required for development of a functional immune system, such as CD154 and CD80.<sup>52–54</sup> Moreover, the microenvironment is potentially responsible for developing an immunosuppressive niche in the lymph nodes and marrow that allows for active immune evasion of the leukemic B cells.<sup>55</sup> Collectively, these cellular defects predispose patients to recurrent opportunistic infections, especially with herpes zoster virus and cytomegalovirus (CMV).<sup>56,57</sup> Defects in class switching of Igs and normal B-cell function also result in progressive hypogammaglobulinemia that predisposes patients to recurrent infections with encapsulated organisms.<sup>58</sup> This may in part be related to the downregulation of CD154 on CLL B cells or through CD95 interaction with its ligand.<sup>59,60</sup> Understanding of these putative pathways has resulted in development of mechanistically relevant targeted therapy.<sup>61</sup>

## ● ROLE OF THE B-CELL RECEPTOR PATHWAY

The B-cell receptor (BCR) plays an integral part in the development and maturation of B cells. Constitutive activation of the BCR is one of the most important survival signals for the propagation of CLL B cells.<sup>62</sup> The surface Ig heterodimer that forms an integral part of the BCR is critical for both antigen-dependent and antigen-independent signaling through the BCR.<sup>63–65</sup> This signal is transduced through a variety of kinases, including LYN (Lck/Yes novel), PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase), SYK (spleen tyrosine kinase), and BTK

(Bruton tyrosine kinase).<sup>64</sup> Their activation results in phosphorylation of phospholipase C-gamma-2 (PLC $\gamma_2$ ) and induction of downstream second messengers that further modulate cell-survival regulators.<sup>35,63</sup> Targeting the various kinases involved in the BCR pathway has resulted in significant improvements in the therapeutic options for this disease. Early results from studies done with BTK, PI3K, and SYK inhibitors have all shown excellent efficacy and tolerability, and these agents are currently being used in various combinations to improve disease outcomes.

BTK was initially characterized as a deficient kinase in patients with X-linked agammaglobulinemia, a disease characterized by a severe immunodeficient state.<sup>66</sup> Specific mutations in BTK result in severe impairments in B-cell development and humoral immunity.<sup>66</sup> Activating mutations of BTK have not been identified in CLL or other cancers. However, CLL B cells tend to have higher levels of BTK that can be induced through the BCR signaling pathway.<sup>67</sup> Efficient targeting of BTK with the irreversible inhibitor ibrutinib results in significant abrogation of downstream survival signaling transduced through this pathway and in the inhibition of cell survival and proliferation.<sup>67</sup> Moreover, ibrutinib irreversibly targets interleukin (IL)-2-inducible T-cell kinase (ITK) in T cells, thus potentiating T-helper type 1 (Th1)-driven immune responses and reversing tumor-induced T-cell anergy.<sup>68</sup>

Similar to BTK, both SYK and PI3K can be induced by both autonomous and antigen-dependent BCR activation and result in providing the critical signals that result in leukemic cell survival and proliferation.<sup>69,70</sup> Similar to ibrutinib, the PI3K isoform delta inhibitor idelalisib antagonizes internal and external survival signals to the CLL cells and results in significant clinical response. Idelalisib is currently approved for the treatment of relapsed CLL.<sup>71</sup> Similar results are observed with SYK inhibitors, which are currently in early-phase clinical trials.<sup>72</sup>

## ● GENETICS OF CHRONIC LYMPHOCYTIC LEUKEMIA

Improved understanding of the genetics of CLL has resulted in significant improvements in our ability to determine the prognosis of this disease and to tailor therapy for our patients. Initial efforts to study the cytogenetic abnormalities in CLL were hindered by the inability of the tumor cells to proliferate *in vitro* for standard metaphase analyses. Improvements in our ability to stimulate the CLL B cells *in vitro* and the development of interphase fluorescent *in situ* hybridization (FISH) techniques have significantly improved and refined the study of cytogenetic abnormalities in this disease.<sup>73</sup> Using these methods, del 13q14 was identified as the most common abnormality in patients with CLL, being present in approximately 50 percent of all patients, followed by trisomy 12, which is present in approximately 15 to 20 percent of patients, and del 11q22.3, which is present in approximately 10 to 15 percent of patients. Other abnormalities that were identified in significant numbers include del 6q21 and del 17p13.1. These abnormalities impart differential prognostic impact on disease outcomes.<sup>74</sup>

Functional studies have been performed to determine the association of these cytogenetic abnormalities to disease physiology. Specifically, deletion in the long arm of chromosome 13 results in a loss of the tumor-suppressor gene ARLTS1 with potential physiologic impact.<sup>75</sup> Moreover, this region also includes genes encoding microRNA (miRNA), including miRNA-15 and miRNA-16.<sup>76</sup> These abundant and evolutionarily conserved short, noncoding miRNA, which range in size from 21 to 25 nucleotides, have the potential to regulate the expression

of a number of different genes at the posttranscriptional level, significantly affecting cell signaling. They are also known to modulate the expression of various pro- and antiapoptotic proteins of significant relevance to CLL B-cell proliferation, including BCL-2 (miRNA-15 and miRNA-16), MCL-1 (miRNA-29), and TCL1 (miRNA-29 and miRNA-181).<sup>76-78</sup> Recent studies have identified miRNA-150 as potentially the most abundantly expressed miRNA in patients with CLL and may be involved in the regulation of BCR signaling and subsequent survival signals.<sup>79</sup> Multiple studies are currently under way to further define the full impact of the role of miRNAs in the pathogenesis of CLL and to develop strategies to modulate their expression and function for therapeutic benefit.

With regard to other cytogenetic abnormalities, trisomy 12 is often found in patients with progressive or relapsed disease or Richter transformation.<sup>80</sup> Numerous genes are affected as a result of the trisomy, and B cells obtained from patients with this abnormality tend to have higher surface expression of CD19, CD20, CD38, and Igs when compared to patients without this abnormality.<sup>80-82</sup> Multiple genetic alterations have also been described in patients with del 11q22.3, but most important, it may involve the loss of the ataxia-telangiectasia mutated (*ATM*) gene, which normally activates p53 and results in either apoptosis or cellular repair in response to cytotoxic stimuli.<sup>83</sup> Moreover, there is a decrease in the expression of miRNA-29 and miRNA-181, which are involved in the downregulation of the T-cell leukemia/lymphoma protein 1A (*TCL1*) oncogene.<sup>84</sup> Patients with del 11q22.3 tend to have more aggressive disease with bulky lymphadenopathy<sup>85</sup>; historically have had a worse prognosis, with disease poorly responsive to conventional nucleoside analogue-based therapy; and have specifically required the addition of alkylating agents like cyclophosphamide to overcome the poor prognostic impact.<sup>86,87</sup>

The del 17p13.1 is present in fewer than 10 percent of patients with CLL and frequently involves the deletion of the tumor protein p53 (*TP53*) gene that encodes the p53 protein, which, as noted previously, is critical for cellular apoptosis or repair specifically in response to cytotoxic stimuli.<sup>88</sup> Patients with this abnormality have rapidly progressive disease with significantly inferior survival outcomes and poor response to therapy.<sup>89</sup> This mutation also confers chemoresistance; consequently, responses to conventional chemotherapy-based regimens have been dismal.<sup>90</sup> These patients also have higher risk of Richter transformation to more aggressive lymphomas over the course of their disease.<sup>89</sup> The inferior outcomes observed in patients with del 11q or del 17p appear to persist in the era of treatment with kinase inhibitors, with patients experiencing a shorter progression-free interval, but are significantly better compared to historic controls.<sup>91,92</sup>

Multiple other cytogenetic abnormalities have been reported from patients with CLL, including del 6q21, those involving the IGH locus on chromosome 14q32, and those involving the *BCL-2* gene located on chromosome 18q21.<sup>93,94</sup> These are generally associated with progressive, relapsed, and aggressive disease and frequently observed in patients with an unmutated immunoglobulin heavy-chain variable (IGHV) region.<sup>93,95</sup>

Other recurring abnormalities in CLL involving *SF3B1* (splicing factor 3B subunit 1), *NOTCH1* (Notch homologue 1, translocation-associated), *MYD88* (myeloid differentiation primary response gene 88), *XPO1* (gene encoding exportin-1), *KLHL6* (Kelch-like protein-6), and *ERK1* (extracellular signal-regulated kinase 1) have been described using whole-exon and whole-genome sequencing with potential prognostic impact.<sup>96-98</sup> With the advent of single nucleotide polymorphism (SNP) arrays, comparative genomic hybridization techniques, and determination of acquired copy number aberrations, more detailed studies can be performed assessing the mechanistic significance and impact of these genetic mutations on prognosis and customizing therapy.



## ● CLINICAL PRESENTATION OF CHRONIC LYMPHOCYTIC LEUKEMIA

CLL is a disease of the elderly, with the median age at diagnosis being 72 years. Most patients are asymptomatic at diagnosis and are diagnosed as a result of incidental finding of lymphadenopathy and lymphocytosis of uncertain etiology as part of an evaluation unrelated to CLL. A vast majority of patients may not have any significant symptoms related to the disease, but some patients may experience mild fatigue or minor limitations in their activities of daily living. A subset of patients may present with recurring infectious complications, especially upper respiratory tract infections. Patients with advanced disease can uncommonly present with drenching night sweats, fevers, and weight loss (B symptoms) and signs and symptoms related to anemia, thrombocytopenia, and lymphadenopathy. The lymphadenopathy typically observed in patients with CLL is generally not fixed or tender and very rarely causes symptoms of organ dysfunction or resulting dependent-limb lymphedema. Patients can have exacerbations of their lymphadenopathy during an acute infectious episode, but this typically returns to baseline upon resolution of the underlying infectious complication.<sup>99</sup> Splenomegaly is seen commonly in patients with CLL with resultant hypersplenism and thrombocytopenia. Significant hepatomegaly because of leukemic infiltration is unusual. CLL infiltration of multiple organs has been described, but this is typically seen in patients with advanced disease and will occasionally cause symptoms. Pulmonary involvement has been observed in patients with a high lymphocyte count and typically presents as an interstitial infiltrate on chest radiography. Chylous and hemorrhagic pleural effusions have also been reported.<sup>100–102</sup> Similarly, leukemic infiltration of the gastrointestinal tract may result in chronic diarrhea or iron-deficiency anemia secondary to chronic bleeding or malabsorption. However, this mucosal infiltration is more commonly seen in patients with mantle cell lymphoma. CLL involvement of the central nervous system is rare and may result in headaches, confusion, meningismus, or cranial nerve palsies.<sup>103</sup> More commonly, these patients are at higher risk for opportunistic infections of the central nervous system because of their deficient immune system. Patients with CLL are also known to have insect bite hypersensitivity.<sup>104,105</sup> Patients typically present with recurrent, erythematous, painful eruptions, usually on the exposed part of the extremities. Evaluation of skin biopsies from these patients reveals a mixed infiltrating population of T cells, B cells, and eosinophils. These populations resolve over time and can be effectively treated with a short course of glucocorticoid.

## ● EVALUATION OF THE PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA

According to the IWCLL-2008 criteria, the diagnosis of CLL requires sustained monoclonal lymphocytosis of greater than 5000 cells/ $\mu$ L of monoclonal B cells.<sup>1</sup> This requires blood flow cytometry for immunophenotyping the B cells, which also reveals the cells to be positive for CD19, dim CD20, and dim surface Ig and negative for CD10, CD79b, and FMC7. A similar disease presentation with no evidence of hematopoietic involvement and with only lymph node involvement by cells of comparable morphology will be classified as small lymphocytic lymphoma. These patients are essentially managed similar to patients with CLL. CLL cells appear as small blue lymphocytes with scant cytoplasm on the Wright-Giemsa staining commonly used for evaluating blood films (Fig. 3–1). Smudge cells are also commonly observed on the blood film, and this results from the mechanical disruption of the cells during the slide preparation process (Fig. 3–2). For an improved evaluation of

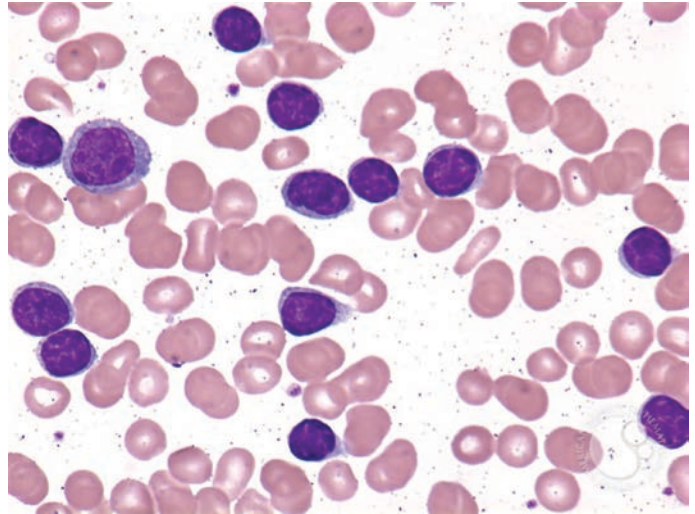


**Figure 3–1.** Typical chronic lymphocytic leukemia blood film. (Reproduced with permission from Ash Image Bank, Peter Maslak, 2013. © the American Society of Hematology.)

the cellular morphology on the blood film, an albumin preparation is sometimes required. Patients can also have large prolymphocytes with prominent nucleoli in the blood, but these lymphocytes must be less than 55 percent of the total lymphocyte population. The anemia is typically normocytic and normochromic, and platelet morphology is typically preserved. A marrow aspirate and biopsy are not required for the vast majority of patients with CLL at initial presentation to establish a diagnosis. However, we do recommend performing a marrow aspirate and biopsy in patients with anemia and thrombocytopenia to evaluate the presence of autoimmune hemolytic anemia and/or immune thrombocytopenia. Marrow biopsy typically shows diffuse marrow involvement with a monotypic population of small lymphocytes. Variability of marrow involvement has been historically used as a potential marker for prognosis but has limited applicability given the availability of more specific and sensitive prognostic markers.<sup>106,107</sup> The red cell precursors and megakaryocytes usually display an unremarkable morphology but do diminish in numbers with progressive disease. Conventional

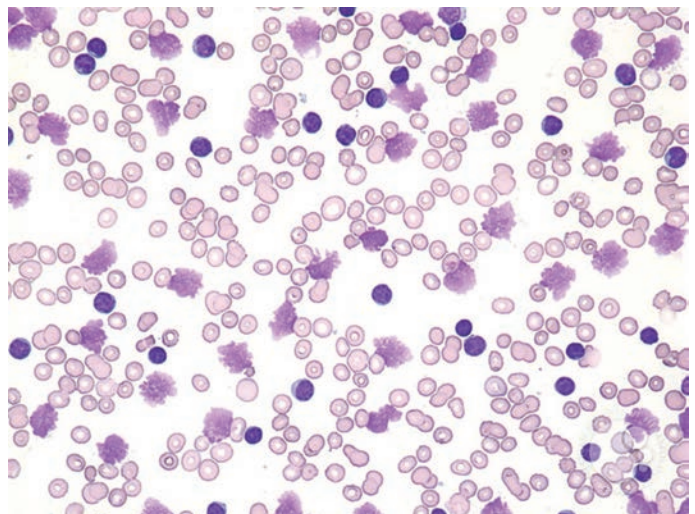


**Figure 3–2.** Typical chronic lymphocytic leukemia blood film with smudge cells. (Reproduced with permission from ASH Image Bank, Peter Maslak, 2010. © the American Society of Hematology.)

stimulated karyotype analysis and interphase FISH cytogenetic study, to evaluate for abnormalities commonly seen in patients with CLL, should be performed on all patients at the time of diagnosis and every time the disease changes character in order to determine the extent of a clonal evolution. Patients with atypical presentations, especially those with absent or low CD23 expression, should have a negative FISH study for t(11;14) to exclude mantle cell lymphoma. Lymph node biopsy is not typically required for further establishing the diagnosis of CLL. Lymph nodes typically show architectural effacement by diffuse infiltration by cells of a similar morphology as observed in the peripheral circulation.

When anemia is present from CLL, patients typically will have normocytic and normochromic anemia, often with thrombocytopenia and lymphocytosis. Patients with a macrocytic anemia or an isolated anemia should have a Coombs test, haptoglobin test, and reticulocyte count performed to rule out autoimmune hemolytic anemia. These patients should also be evaluated for vitamin B<sub>12</sub> and folic acid deficiencies, and malabsorption or gastrointestinal bleeding from CLL involvement should be ruled out.

Patients with CLL will frequently have hypogammaglobulinemia with a decrease in the serum concentration of IgG, IgA, and IgM. The degree of hypogammaglobulinemia correlates with progressive disease and predisposes patients to recurrent sinopulmonary infections with encapsulated organisms. T-cell defects, which increase the risks of viral infections, have also been described; however, this is not routinely assessed at the time of initial presentation. A small percentage of patients also have monoclonal gammopathy with IgM or IgG or light-chain monoclonal paraproteinemia, which can be detected on serum protein electrophoresis and immunofixation. Excessively high heavy-chain paraproteinemia can result in symptoms related to hyperviscosity, as seen in patients with Waldenström macroglobulinemia, and should be managed as such.<sup>108–110</sup> The presence of a monoclonal paraproteinemia and/or hypogammaglobulinemia may be related to inferior survival outcomes in patients with advanced disease stage, but not necessarily in patients with early-stage disease.<sup>111,112</sup>

## ● PROGNOSTIC MARKERS

### CYTOGENETICS AND FLUORESCENCE IN SITU HYBRIDIZATION

All patients must undergo a comprehensive prognostic evaluation at the time of initial presentation. This allows the clinician to explain the specific disease characteristic to the patient and also helps the patient with the emotional adjustment process that the patient has to go through when initially diagnosed with this disease. All patients should undergo conventional karyotype analysis and stimulated interphase FISH on either blood or marrow aspirate. The minimum FISH panel should include assessment for del 17p13, del 11q23, trisomy 12, and del 13q14 and for t(11;14) in selected patients suspected of having mantle cell lymphoma. Conventional stimulated karyotype analysis is helpful in identifying the global structural abnormalities in chromosomes, especially of chromosomes 14, 3, and 6, that cannot be routinely detected on FISH analysis.<sup>113</sup> Together, these assays have strong prognostic significance with regard to treatment-free and overall survival (OS; Table 3–1).<sup>114</sup> Patients with CLL acquire additional cytogenetic abnormalities, as detected by stimulated karyotyping and FISH analysis, with disease progression and especially after chemotherapy. This “clonal evolution” is predominantly observed in patients with unmutated IGHV and portends poor survival and inferior response to therapy.<sup>115</sup> We therefore recommend repeating the stimulated karyotyping and FISH studies prior to initiation of a new line of treatment.

**TABLE 3–1.** Survival Outcomes and Time to First Treatment Based on FISH Cytogenetics and IGHV Status

| Prognostic Variables    | Median Survival (Months) | Median Time to First Treatment |
|-------------------------|--------------------------|--------------------------------|
| 13q– (sole)             | 133                      | 92 months                      |
| Trisomy 12              | 114                      | 33 months                      |
| Normal                  | 111                      | 49 months                      |
| 11q–                    | 79                       | 13 months                      |
| 17p–                    | 32                       | 9 months                       |
| Unmutated (≥98 percent) | 89                       | 3.5 years                      |
| Mutated (<98 percent)   | >152                     | 9.2 years                      |

FISH, fluorescence *in situ* hybridization; IGHV, immunoglobulin heavy-chain variable region.

### IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION MUTATION ANALYSIS

The assessment of IGHV somatic mutation by a polymerase chain reaction–based assay has been shown to be an extremely reliable and important prognostic tool for patients with CLL. Patients with less than 2 percent homology in their nucleotide sequence as compared to consensus germline sequence are considered unmutated.<sup>116</sup> Patients with a mutated IGHV, which is present in approximately 60 percent of patients with CLL, have a significantly prolonged treatment-free interval, longer remission durations, and OS.<sup>117</sup> These patients also have a very low incidence of clonal evolution or transformation to an aggressive histology.<sup>114,118</sup> The IGHV mutation status does not vary over time and does serve as a reliable marker for predicting long-term disease outcomes.<sup>119</sup> The only known exception to the mutation rule currently is the presence of IGHV 3–21 somatic mutation, which may confer an aggressive phenotype similar to leukemic cells from patients with unmutated IGHV at least in a subset of patients.<sup>120,121</sup>

### ZETA-CHAIN–ASSOCIATED PROTEIN KINASE OF 70 KDA AND ITS METHYLATION STATUS

Zeta-chain–associated protein kinase of 70 kDa (ZAP-70) is an intracellular tyrosine kinase that is typically associated with T-cell development and T-cell receptor (TCR) signaling. Expression of ZAP-70 in CLL B cells provides a survival advantage through intrinsic and extrinsic signals mediated through the BCR.<sup>122</sup> Cytoplasmic assessment of ZAP-70 in CLL B cells by flow cytometry correlates strongly with IGHV mutational status and clinical outcomes, with an expression of 20 percent or more predictive of poor outcomes.<sup>123</sup> The assessment of ZAP-70 by flow cytometric testing has been plagued with several issues, including lack of reproducibility and reliability of the reagents. Consequently, the National Comprehensive Cancer Network (NCCN) guidelines do not recommend the routine use of ZAP-70 as a prognostic marker outside of clinical trials. Given the stability of DNA and epigenetic modification by methylation, investigators have also sought to assess ZAP-70 expression by the absence of promoter methylation.<sup>124</sup> Methylation analysis of select proximal 5′ regions of the ZAP-70 gene correlates very strongly with expression of ZAP-70 and has been established as an important and reliable prognostic marker with regard to predicting time to treatment and OS.<sup>125,126</sup> This assay can be done by pyrosequencing, with significant reproducibility among different laboratories.<sup>126</sup>



## CD38 EXPRESSION

CD38 is a 45-kDa transmembrane glycoprotein that can be detected on the surface of CLL B cells by flow cytometry; a level of expression greater than 30 percent correlates strongly with progression-free survival (PFS).<sup>118</sup> Newer reports, however, suggest that an even lower level of CD38 expression might also have a prognostic impact.<sup>127,128</sup> CD38 appears to be involved in cellular metabolism by synthesizing cyclic adenosine diphosphate (cADP)-ribose from nicotinic acid adenine (NAD),<sup>129</sup> and its activity and expression correlate with proliferation of the lymphocytes and progressive disease as demonstrated by their high Ki-67 proliferation index.<sup>130</sup> CD38 expression also changes with disease progression and the assessment of the extent of expression of CD38 is based on nonstandardized, subjective parameters.<sup>131</sup>

## CD49D EXPRESSION

CD49d can also be used as a reliable predictive marker. CD49d is a surface subunit of the integrin heterodimer that is involved in promoting survival of the CLL cells through growth signals derived from the microenvironment.<sup>132,133</sup> Patients with 30 percent or greater cells expressing CD49d by flow cytometry are considered to be positive and constitute a group of patients with an aggressive disease course and inferior survival.<sup>134</sup>

## OTHER PROGNOSTIC MARKERS

Serum lactate dehydrogenase (LDH) and  $\beta_2$ -microglobulin are readily available, validated markers of disease aggressiveness and prognosis. Specifically,  $\beta_2$ -microglobulin is an independent prognostic marker for remission duration, PFS, and OS, and a higher level is observed in patients with advanced and extensive disease.<sup>135–137</sup> Elevated LDH is associated with more aggressive disease and with Richter syndrome (RS). Lymphocyte doubling time could also be used as a tool to determine the prognosis of CLL. Patients with a lymphocyte doubling time of 12 months or less have worse OS and treatment-free survival.<sup>138</sup> Other reasons for transient elevation in lymphocyte count should be ruled out before making the determination of lymphocyte doubling time. Thymidine kinase is an important intracellular enzyme; the soluble form can be detected in patients with CLL and predicts advanced stage and progressive disease. However, the assay is not widely available, and the test is rarely used in routine clinic care.<sup>139–141</sup> Over the years, various other serum proteins have been found to be associated with various measures of disease outcomes, including soluble factors like CD23,<sup>142</sup> CD44,<sup>143</sup> vascular cell adhesion molecule (VCAM)-1,<sup>144</sup> CD27,<sup>144</sup> and matrix metalloproteinase (MMP)-9,<sup>145</sup> IL-6,<sup>146</sup> and IL-8.<sup>147</sup> However, none of these is routinely used for clinical decision making (Table 3–2).

Various miRNAs have also been validated as useful prognostic markers. miRNAs are noncoding RNAs that are 19 to 25 nucleotides in length and modulate mRNA translation and synthesis of various proteins. miRNA-15a and miRNA-16-1 were the first ones to be identified as underexpressed in CLL patients' B cells.<sup>76</sup> Genes for these miRNAs are located in the deleted region of chromosome 13q14 and modulate the expression of the antiapoptotic bcl-2 protein, which is overexpressed in patients with CLL and other B-cell lymphoproliferative disorders.<sup>148</sup> Similarly, miRNA-34c is involved in patients with del 11q23 and regulates the expression of ZAP-70 and other proteins involved in the TP53 pathway.<sup>149</sup> Using mass array methods, miRNA profiles have also been found to be predictive for disease progression, fludarabine resistance, and clinical outcomes.<sup>150,151</sup>

**TABLE 3–2.** Outcomes of Selected Prognostic Factors

|                              | Favorable Outcome                   | Unfavorable Outcome     |
|------------------------------|-------------------------------------|-------------------------|
| Lactate dehydrogenase        | Low or normal                       | Elevated                |
| Lymphocyte doubling time     | >12 months                          | ≤12 months              |
| Thymidine kinase activity    | Low or normal                       | Elevated                |
| $\beta_2$ -Microglobulin     | Low or normal                       | Elevated                |
| Soluble CD23 levels          | Low or normal                       | Elevated                |
| CD38 expression              | <30 percent                         | >30 percent             |
| Interphase FISH cytogenetics | Normal<br>Trisomy 12<br>13q– (sole) | 11q–<br>17p–            |
| IGHV mutational status       | Mutated (<98 percent)               | Unmutated (≥98 percent) |
| CD49d expression             | <30 percent                         | >30 percent             |

FISH, fluorescence *in situ* hybridization.

## STAGING FOR CHRONIC LYMPHOCYTIC LEUKEMIA

The Rai<sup>152</sup> and Binet<sup>153</sup> staging systems have been used for a long time for patients with CLL (Tables 3–3 and 3–4, respectively). These easy-to-use staging systems are based on assessment of disease burden as determined by lymphadenopathy and splenomegaly demonstrated on physical examination and the presence of cytopenias. Further modifications and development of the Binet system established low-risk (stage 0: lymphocytosis only), intermediate-risk (stages 1–2: lymphocytosis with lymphadenopathy and hepatosplenomegaly), and high-risk groups of patients with CLL (stages 3–4: lymphocytosis with anemia and thrombocytopenia).<sup>153</sup> These classification systems provided an estimate of median OS of 150, 90, and 19 months, respectively, and helped classify patients for subsequent therapeutic intervention. The staging systems still remain relevant and complementary to molecular testing in the modern era.<sup>154</sup>

Marrow aspirate and biopsy are not routinely required for the initial diagnosis and management of the patient with CLL. Marrow biopsy is especially helpful in determining the etiology of thrombocytopenia and anemia, which can frequently be related to concomitant autoimmune processes. It may also be helpful in determining the extent of involvement by large prolymphocytes and in establishing the diagnosis of prolymphocytic leukemia. A diffuse pattern of involvement of the biopsy specimen by characteristic small lymphocytes is also associated with a worse prognosis than interstitial or nodular involvement.<sup>106,155</sup> Ideally, a marrow biopsy is performed at the start of therapy to document the disease extent and to detect any atypical features.

Computed tomographic (CT) scans are generally not required for the routine initial evaluation of patients with CLL, and conventional staging systems rely on physical examination findings. Similarly, routine CT scans for the serial evaluation of disease extent have no role in patients with CLL. CT scans should be performed in patients with symptomatic disease and prior to starting therapy. Similarly, a positron emission tomographic (PET) scan has no role in the routine management of patients with CLL. CLL lymph nodes are fluorodeoxyglucose

**TABLE 3-3.** Modified Rai Clinical Staging System

| Stage at Diagnosis | Risk Level   | Rai Stage at Diagnosis                                                    | Patients Never Requiring Therapy (%) | Median Survival (Months) |
|--------------------|--------------|---------------------------------------------------------------------------|--------------------------------------|--------------------------|
| 0                  | Low          | Lymphocytosis $>5 \times 10^9/L$ only                                     | 59                                   | 150                      |
| 1                  | Intermediate | Lymphocytosis + lymph node (LN) enlargement                               | 21                                   | 101                      |
| 2                  |              | Lymphocytosis + spleen/liver (S/L) enlargement $\pm$ LN                   | 23                                   | 71                       |
| 3                  |              | Lymphocytosis + anemia (with hemoglobin $<11$ g/dL) $\pm$ LN or S/L       | 5                                    | 19                       |
| 4                  | High         | Lymphocytosis + thrombocytopenia ( $<100 \times 10^{12}/L \pm$ LN) or S/L | 0                                    | 19                       |

(FDG) nonavid; however, FDG-PET scanning can be used for the identification of patients with Richter transformation with a high sensitivity and negative predictive value.<sup>156,157</sup>

Based on disease extent, characteristics and prognostic markers, various nomograms have been developed and validated for use in patients with CLL. These nomograms provide a robust predictive tool for outcomes and incorporate various aspects of the CLL patient and disease and can be used for standardized risk assessment.<sup>136,137,158</sup>

The CLL–International Prognostic Index (CLL-IPI) is a similar prognostic tool that identified five independent prognostic factors: TP53 status (no abnormalities vs. del 17p or TP53 mutation or both); IGHV mutational status (mutated vs. unmutated); serum  $\beta_2$ -microglobulin concentration ( $\leq 3.5$  mg/L vs.  $>3.5$  mg/L); clinical stage (Binet A or Rai 0 vs. Binet B–C or Rai 1–4); and patient's age ( $\leq 65$  years vs.  $>65$  years). The presence of del 17p and/or aberrant TP53 was the variable with the most adverse impact on OS.<sup>158a</sup> With the utility of the CLL-IPI, CLL patients were stratified into four risk groups that predicted 5-year OS of 93.2 percent for low-, 79.3 percent for intermediate-, 63.3 percent for high-, and 23.3 percent for very high-risk disease.

## TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA

Treatment of patients with CLL is initiated at the time of symptomatic progressive disease. The specific criteria for initiating therapy have been detailed in the IWCLL-2008 guidelines.<sup>1</sup> This recommendation is primarily based on older studies that failed to demonstrate a survival advantage in patients treated early in the course of disease.<sup>159,160</sup>

These results were validated by a large study of fludarabine treatment in patients with early-stage disease conducted by the German CLL study group, which failed to show a survival advantage with early treatment using conventional chemotherapeutic agents.<sup>161</sup> Trials are currently under way with kinase inhibitors to determine if early intervention can alter the natural history of the disease. We recommend initiating treatment when patients fulfill the IWCLL-2008 criteria for treatment, regardless of the prognostic factors.

Patients with autoimmune complications of CLL can be managed accordingly with steroids and immunosuppressive therapies prior to proceeding with definitive therapy for the underlying disease. Patients with CLL rarely exhibit evidence of leukostasis resulting from profound leukocytosis; therefore, an elevated white cell count should not be used as a sole criterion for initiating treatment. Similarly, hypogammaglobulinemia should not be used as a reason to treat the disease. Periodic intravenous Ig infusions can be used in patients with hypogammaglobulinemia and recurrent life-threatening infections with encapsulated organisms. The IWCLL-2008 criteria should also be used when determining the timing of therapy for patients with relapsed disease.

One of the most important factors to consider prior to initiating treatment is the functional state of the patient. Historically, age cutoff has been successfully used in developing specific therapies. Because the median age of diagnosis for CLL is 72 years, the vast majority of patients treated in the community are older and with multiple comorbid conditions. Unfortunately, there are limited data available in this patient population, and until recently they had very limited options for therapy. Most of the clinical trial participants have been younger patients in their 50s and 60s. The gradual functional decline, decrease in organ function, especially renal and hepatic function, and an increase in the comorbid

**TABLE 3-4.** Binet Clinical Staging System

| Stage at Diagnosis | Equivalent Rai Staging | Rai Stage at Diagnosis                                                                                                                                              | Proportion of Patients (%) | Median Survival (Years) |
|--------------------|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|-------------------------|
| A                  | 0–2                    | Lymphocytosis $>5 \times 10^9/L$ only with $<3$ enlarged nodal areas*; no anemia, no thrombocytopenia                                                               | 15                         | 12+                     |
| B                  | 1–2                    | Lymphocytosis $>5 \times 10^9/L + \geq 3$ enlarged nodal areas*; no anemia, no thrombocytopenia                                                                     | 30                         | 7                       |
| C                  | 3–4                    | Lymphocytosis $>5 \times 10^9/L +$ anemia (hemoglobin $<10$ g/dL) or thrombocytopenia ( $<100 \times 10^{12}/L$ ) regardless of the number of enlarged nodal areas* | 55                         | 2                       |

\*Nodal areas counted as one each of the following: axillary, cervical, inguinal lymph nodes, whether unilateral or bilateral; spleen, and liver.



conditions in the majority of patients older than age 65 years significantly increase the risks and toxicities of conventional chemotherapeutic regimens, especially nucleoside analogues. To address these issues, different approaches are being used to treat patients older than 65 years versus younger patients. A cumulative illness rating scale (CIRS) has been proposed and used primarily by the German CLL study group. It allows patients to be stratified based on an aggregate score derived from multiple factors, including age, comorbid conditions, and organ function.<sup>162</sup>

## DEVELOPMENT OF CHEMOTHERAPY FOR THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA

### *Alkylating Agents*

Chlorambucil has been used as the prominent alkylating agent for the treatment of CLL for the last 60 years. Chlorambucil is taken orally and is generally well tolerated but does have multiple side effects, including nausea, vomiting, and cytopenias. Various doses and schedules have been used with different responses and tolerability profiles. Older studies compared chlorambucil to conventional high-grade lymphoma therapy, including cyclophosphamide, vincristine, and prednisone (CVP) and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), and found no improvement in survival outcomes with the use of high-grade lymphoma-like therapies.<sup>159</sup> Multiple doses of chlorambucil have been evaluated in various clinical trials with differing but similarly modest overall response rate (ORR) and PFS. No single-dosing regimen has been shown to be superior to another. One dosing regimen that has been used in cooperative group studies is a 40 mg/m<sup>2</sup> oral dose every 28 days for 12 cycles.<sup>163</sup> An argument, however, can be made that escalating the dose of chlorambucil in younger patients might result in a higher response rate.<sup>164</sup> Despite its ease of use and reasonable tolerability, chlorambucil is not commonly used today because of the availability of better and potentially safer alternatives. Moreover, responses observed with chlorambucil are modest and not durable.<sup>165</sup> Nevertheless, chlorambucil is potentially an option for treatment of elderly patients with multiple comorbidities and limited other options for treatment. It should, however, not be used for asymptomatic patients with early-stage disease.<sup>160</sup>

Cyclophosphamide is also approved for the use in patients with CLL and has moderate efficacy and reasonable tolerability. Care should be taken to avoid nighttime dosing, and aggressive hydration should be encouraged to avoid hemorrhagic cystitis.<sup>166</sup> Low-dose etoposide, either as a single agent or in combination with cladribine, also shows modest responses in patients with relapsed and refractory disease. Treatment with etoposide is associated with significant myelosuppression and resultant infectious complications.<sup>167,168</sup>

Bendamustine was approved for the treatment of patients with CLL in 2008. This was based on a phase III trial that demonstrated the superior efficacy of bendamustine as compared to chlorambucil with regard to response rate and PFS.<sup>169</sup> Structurally, bendamustine has features common with alkylating agents and purine analogues, but its activity is primarily derived from the alkylating agent moiety.<sup>170</sup> Bendamustine appears to be generally better tolerated than fludarabine but causes significant myelosuppression, requiring dose reductions, especially in elderly and infirm individuals. Tolerability is better in patients with impaired renal function since excretion is primarily through the feces.<sup>171</sup>

### *Nucleoside Analogues*

Nucleoside analogues have also been used for the treatment of patients with CLL for the last 25 to 30 years. Fludarabine has been the most commonly used agent from this class of drugs. Fludarabine has moderate

activity especially in younger patients with good nutritional status and with untreated, early-stage disease.<sup>172</sup> After demonstrating promising activity as a single agent in early-phase clinical trials, fludarabine was compared to chlorambucil in a randomized phase III study for the initial treatment of patients with CLL. Patients treated with fludarabine demonstrated improvement in PFS and OS compared to chlorambucil, albeit with a higher rate of infectious complications; however, all patients ultimately had recurrent disease.<sup>163</sup> Similar to chlorambucil, fludarabine was also more effective compared to combination chemotherapy regimens like cyclophosphamide, doxorubicin, and prednisone (CAP).<sup>173</sup> However, PFS and OS advantage was not shown in another randomized trial of elderly patients older than age 70 years treated with fludarabine on chlorambucil despite the higher overall and complete response (CR) rate in the fludarabine-treated patients.<sup>174</sup>

The use of fludarabine and similar nucleoside analogues is associated with significant hematologic and immunologic toxicities. Patients can experience prolonged cytopenias, especially neutropenia, which can result in a significant increase in the risk of infectious complications. These drugs are also exquisitely toxic to T cells, especially to CD4+ T cells. This effect may last for an extended period of time and predisposes patients to acquiring opportunistic infections.<sup>175,176</sup> Neurologic toxicities have also been observed in patients receiving the usual dose of fludarabine.<sup>177</sup> Patients treated with fludarabine occasionally develop autoimmune hemolytic anemia. In this situation, further use of fludarabine is contraindicated.<sup>178–180</sup> Fludarabine is also poorly tolerated in patients with compromised renal function as a significant percentage of the metabolites are cleared via the renal system. Hemodialysis is a useful tool to limit fludarabine toxicity in patients who develop acute renal failure while receiving treatment.<sup>181</sup> Moreover, fludarabine treatment in patients older than age 65 years was poorly tolerated and did not result in improvements in PFS and resulted in a trend toward inferior OS outcome when compared to chlorambucil.<sup>174</sup>

Other nucleoside analogues used in the treatment CLL include cladribine and pentostatin, which have similar outcomes and toxicities as fludarabine.<sup>182–185</sup> A phase III trial comparing fludarabine, cladribine, and chlorambucil showed similar ORR and CR rate with all three agents, but median PFS was superior with cladribine (25 months) versus 10 and 9 months with fludarabine and chlorambucil, respectively.<sup>186</sup> No advantages, however, were observed in OS. Cytarabine has also shown modest activity, especially in combination with oxaliplatin, fludarabine, and rituximab (OFAR regimen) in patients with refractory disease and patients with Richter transformation.<sup>187,188</sup>

## COMBINATION CHEMOTHERAPY

Multiple chemotherapeutic combinations have been studied in patients with CLL. One of the earlier combinations to be used was chlorambucil and prednisone, with ORR of approximately 80 percent and CR rates of approximately 10 to 15 percent.<sup>165,189</sup> This regimen was also found to be as effective as other combination regimens like CVP; cyclophosphamide, melphalan, and prednisone (CMP); CAP; or CHOP.<sup>159,190–192</sup> A meta-analysis comparing lymphoma-like combination therapies found no improvements in survival outcomes over chlorambucil alone.<sup>159</sup> High-dose combination therapies like CAP, CHOP, and CVP have no role in the routine management of patients with CLL.

Given the improvement in outcomes with single-agent fludarabine as compared to chlorambucil, multiple combinations were developed with fludarabine to improve on its efficacy. Both combinations, of fludarabine and chlorambucil and of fludarabine and prednisone, were found to be similar to fludarabine and not developed further.<sup>172,193,194</sup> Fludarabine was combined with cyclophosphamide (FC) and resulted in encouraging responses even in patients with heavily pretreated disease.<sup>195</sup>

Multiple phase III trials were subsequently conducted in predominantly younger patients, comparing fludarabine with FC and revealed an ORR of 74 to 94 percent with CR rates of 23 to 38 percent and median PFS of 33 to 48 months, all significantly better with the combination.<sup>87,196,197</sup> Early toxicities were mostly related to cytopenias secondary to myelosuppression, and the resultant increase in infectious complications resulted in a slight dose adjustment; a dose of fludarabine 25 to 30 mg/m<sup>2</sup> for 3 days and cyclophosphamide 250 mg/m<sup>2</sup> for 3 days every 28 days for six cycles was used for subsequent studies. Notably, the combination of FC was the first regimen that was able to overcome the adverse prognostic impact of del 11q.<sup>86</sup>

Similarly, other combinations of alkylating agents with nucleoside agents have been tested with varying success. These include cladribine and prednisone, which in combination was shown to be better than chlorambucil and prednisone in terms of responses, but without improvements in OS, and with a higher incidence of infectious complications in the cladribine-treated arm.<sup>198,199</sup> Similarly, the addition of cyclophosphamide and prednisone to cladribine resulted in higher responses but more myelosuppression and related complications.<sup>200–202</sup> Pentostatin was also combined with cyclophosphamide and resulted in an ORR of 74 percent and CR rates of 17 percent in patients with fludarabine refractory disease.<sup>203</sup> Pentostatin in combination with chlorambucil and prednisone also resulted in promising responses but was extremely immunosuppressive and resulted in an unacceptably high incidence of infectious complications.<sup>204</sup>

Fludarabine was also combined with mitoxantrone without significant improvements in outcome. Although mitoxantrone appeared to improve outcomes when added to cladribine, it came at the cost of significant toxicity.<sup>205,206</sup> Likewise, the combination of fludarabine, cyclophosphamide, and mitoxantrone resulted in an ORR of 78 percent and CR rates of 50 percent in patients with relapsed disease and an ORR of 90 percent with a CR rate of 38 percent in patients with previously untreated disease. The major toxicity was myelosuppression.<sup>206,207</sup>

## ANTIBODY THERAPY

The advent of antibodies for the treatment of patients with CLL has been a major advance in the management of this disease with the first true consistent evidence of improving survival. Numerous antibodies targeting different receptors have been developed and are at various stages of development. Four antibodies are currently approved for routine management. Unfortunately, one of these, alemtuzumab (an anti-CD52 antibody), is no longer actively marketed for this indication and thus is not discussed in this chapter.<sup>208–219</sup>

### CD20-Targeting Antibodies

**Rituximab** Rituximab is a chimeric, murine, CD20-targeting, monoclonal antibody that has been extensively used for the treatment of patients with CD20+ lymphoid malignancies. CD20 is a calcium channel that interacts with the BCR complex, is ubiquitously expressed on B-cell non-Hodgkin lymphomas, and has weak expression on CLL cells. Rituximab exerts its efficacy through direct complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC).<sup>220–222</sup>

The dose and schedule of treatment with rituximab were determined empirically and have since been modified repeatedly. Initial trials were performed with four weekly infusions at 375 mg/m<sup>2</sup> and showed limited efficacy in patients with CLL.<sup>223,224</sup> Responses were higher when higher doses (up to 2250 mg/m<sup>2</sup>) or dose-dense regimens (375 mg/m<sup>2</sup> three times a week) were used but were primarily limited to the blood and nodal areas.<sup>225,226</sup> Nonetheless, these studies established the efficacy of rituximab and supported combination trials with chemoimmunotherapy where its activity has been most impactful.

Rituximab is generally tolerated very well, with the most common toxicity being infusion reactions, which are predominantly observed with the first dose. These are generally mild fevers or chills but occasionally may result in serious reactions that mimic severe allergic or anaphylactic reactions or cytokine release syndrome. The infusion reactions can be minimized with the routine use of prophylactic acetaminophen, antihistamine, and corticosteroids, or glucocorticoid and by slowing the infusion rate. Patients may also experience transient, severe thrombocytopenia, the mechanism of which is poorly understood. Therefore, rituximab should be used with caution in patients with a preexisting thrombocytopenia. Another important and potentially severe toxicity is tumor lysis syndrome, which is generally observed in patients with a high circulating peripheral lymphocyte count. These patients should be monitored closely and should receive prophylactic hydration, allopurinol, and electrolyte monitoring during and after the infusion. Other uncommon toxicities include delayed neutropenia, hepatitis B reactivation, interstitial pneumonitis, rash, and serum sickness. Patients with a prior history of hepatitis B infection should receive monitoring for reactivation while being treated with rituximab or similar agents, with rapid implementation of antiviral therapy if reactivation is observed. Fatal cases of progressive multifocal leukoencephalopathy from JC polyomavirus infection have also been reported with the use of rituximab and similar monoclonal antibodies. These infections typically occur during treatment or soon after, with virtually all cases observed during the first year posttherapy.<sup>227,228</sup>

**Ofatumumab** Ofatumumab is a fully human, type 1, IgG<sub>1</sub>, CD20-targeting, monoclonal antibody that binds more effectively to a different epitope of CD20 than rituximab.<sup>229</sup> *In vitro* it was shown to have improved CDC and ADCC as compared to rituximab.<sup>230,231</sup> Ofatumumab is given as a test dose of 300 mg followed by eight weekly intravenous infusions of 2000 mg, after which patients can go on a maintenance schedule of four monthly infusions of 2000 mg. The half-life of ofatumumab is 21 days, but the B-cell-depleting effects may last for up to 7 months after the last infusion.<sup>232</sup>

Early results with ofatumumab as a single agent in patients who were refractory to alemtuzumab and/or fludarabine showed encouraging ORR of 58 percent in the double-refractory group and 47 percent in the fludarabine-refractory cohort with bulky disease. Responses were all partial except for one CR in the fludarabine-refractory group. Response was also short lived, and the median duration of response was 7 months in the fludarabine-refractory group and 5.6 months in the double-refractory group, with most patients progressing during treatment.<sup>233</sup> These results led to the approval of ofatumumab in patients with relapsed disease refractory to alemtuzumab and/or fludarabine in 2009. Subsequent studies compared ofatumumab (300 mg on day 1 followed by eight weekly infusions of 1000 mg, followed by 1000 mg on day 1 of subsequent 28-day cycles, for a maximum of 12 cycles) given in combination with oral chlorambucil (10 mg/m<sup>2</sup> on days 1 to 7 of each 28-day cycle) versus chlorambucil alone in patients with previously untreated CLL who required treatment and were not considered candidates for conventional chemoimmunotherapy. The combination resulted in an ORR of 82 percent versus 68 percent with chlorambucil alone. However, CR rates were 12 percent versus 1 percent, and median duration of response was 22 months versus 13 months in the combination versus chlorambucil arms, respectively.<sup>234</sup> Based on these results, the combination of ofatumumab and chlorambucil was granted approval in 2014 for the treatment of previously untreated patients with CLL for whom fludarabine-based therapy is considered inappropriate.

Ofatumumab has also been studied in the upfront setting in combination with FC in smaller phase II studies of a relatively young patient population (median age: 56 years). This study revealed an ORR of 75 percent with 41 percent CRs.<sup>235</sup> The combination of ofatumumab

with pentostatin and cyclophosphamide resulted in a 96 percent ORR with 46 percent complete remission rate.<sup>236,237</sup> This compares favorably to the results seen with the combination of rituximab and chemotherapy, but large multi-institution randomized trials are lacking. In patients who are not candidates for conventional chemoimmunotherapy, a phase III study compared the efficacy of the combination of ofatumumab and chlorambucil to chlorambucil alone and established the superiority of ofatumumab in this setting.<sup>234</sup> However, a major criticism of this study is the lack of a direct comparison with rituximab, which has limited the use of this approach.

Similarly, maintenance ofatumumab for 2 years after second- or third-line therapy was shown to improve PFS by 14 months over observation alone.<sup>237a</sup> However, it does not impact OS, and time to next treatment was shorter, suggesting its impact is to delay relapse rather than achieve deeper remissions.

Ofatumumab is generally well tolerated, with the most common reaction being an infusion-related reaction that typically occurs with the first infusion and includes fevers, rash, fatigue, chills, and diaphoresis. These reactions tend to get better with subsequent infusions. Infectious complications are similar to those reported with other CD20 monoclonal antibodies.

**Obinutuzumab** Obinutuzumab is a CD20-targeting antibody that was approved in 2014 in combination with chlorambucil for the initial treatment of patients with CLL. Obinutuzumab is a fully humanized, type II, IgG<sub>1</sub> antibody<sup>238</sup> with additional structural modifications that explain its enhanced activity. It binds selectively to the extracellular domain of CD20 with reduced internalization. This persistence of the antibody on the cell surface along with a fucosylation in its Fc region allow for enhanced ADCC through robust engagement of Fc-gamma receptor type III on effector cells. Another modification in the hinge region allows for more potent direct cytotoxicity.<sup>239–241</sup> Together, these modifications translate into higher efficacy as compared with rituximab in both preclinical and clinical studies.<sup>239–243</sup>

Obinutuzumab in combination with chlorambucil was compared to rituximab and chlorambucil and chlorambucil alone in patients with untreated CLL in the CLL-11 trial conducted by the German CLL study group.<sup>244</sup> Patients had a median age of 73 years, which is closer to the median age of 72 years at diagnosis for CLL patients and significantly higher than the median ages of 58 to 62 years that have historically been the population that has been enrolled in chemotherapy-based clinical trials for CLL. More important, these patients had clinically meaningful comorbid conditions. Treatment of this patient population has historically been challenging, and no prior chemotherapeutic option, including fludarabine, has improved survival outcomes as compared to chlorambucil alone.<sup>174</sup> The combination of obinutuzumab and chlorambucil improved ORRs (77.3 percent [obinutuzumab and chlorambucil] vs. 65.7 percent [rituximab and chlorambucil] vs. 31.4 percent [chlorambucil]); CR rates (22.3 percent [obinutuzumab and chlorambucil] vs. 7.3 percent [rituximab and chlorambucil] vs. 0 percent [chlorambucil]); and median PFS (26.7 months [obinutuzumab and chlorambucil] vs. 16.3 months [rituximab and chlorambucil] vs. 11.1 months [chlorambucil]). Obinutuzumab with chlorambucil also prolonged OS over that observed with chlorambucil. Notably, the combination also resulted in significant improvement in the rate of minimal residual disease (MRD)–negative status in both marrow (19.5 percent vs. 2.6 percent) and blood (37.7 percent vs. 3.3 percent) as compared to rituximab and chlorambucil.

Obinutuzumab is generally well tolerated but has a high incidence of infusion reactions, which are seen primarily with the first infusion. Unlike rituximab or ofatumumab, for which infusion events occur 1 to 2 hours into therapy, those with obinutuzumab typically occur within the first 5 to 10 minutes of starting therapy. These may be minimized by

a test dose, slower infusion rate, and prophylactic steroids, acetaminophen, and antihistamines. Tumor lysis is also seen in a small number of patients, reflecting the higher efficacy of obinutuzumab. Hematologic toxicities, like neutropenia and thrombocytopenia, are also observed. All CD20-targeting antibodies increase the risk of hepatitis B virus reactivation and progressive multifocal leukoencephalopathy.

The activity of obinutuzumab in patients with untreated CLL is very exciting, and this antibody is being combined with other novel agents and chemotherapy to further improve outcomes in patients with CLL.

### Other Antibodies and Antibody-Like Compounds

Multiple antibodies targeting various antigens like CD19 and CD37 are at various stages of development for the treatment of CLL.<sup>245–247</sup> Advances in antibody-manufacturing technology are enabling us to synthesize more potent and bispecific antibodies, like blinatumomab, which has already shown promising activity in patients with acute lymphoblastic lymphoma (ALL) and CLL.<sup>248</sup>

## GLUCOCORTICOIDS

Glucocorticoids are effective agents in the management of patients with relapsed CLL and especially patients with del 17p and fludarabine-refractory disease. High-dose methylprednisolone, either as a single agent or in combination with multiple other chemotherapeutic agents, produced sustained responses in the majority of patients with refractory and high-risk disease.<sup>249</sup> High-dose methylprednisolone at 1 g/m<sup>2</sup> for 5 days with weekly rituximab for three cycles induces response rates as high as 93 percent with a CR of 36 percent.<sup>250</sup> Therapy, however, is complicated by significant hyperglycemia, fluid retention, and immune suppression, resulting in increased incidence of opportunistic infections requiring close followup and aggressive supportive care with prophylactic antibiotics. The addition of weekly rituximab and decreasing the duration of methylprednisone dose to 3 days resulted in similar responses and reduction in the incidence of adverse events.<sup>250,251</sup> Similar results were observed when methylprednisolone was substituted for dexamethasone 40 mg weekly or every 2 weeks for 4 days.<sup>252</sup> Glucocorticoids at lower doses are also very useful in the management of patients with autoimmune complications as a result of their CLL.

## CHEMOIMMUNOTHERAPY

The combining of chemotherapy with targeted antibodies has been a major advance in the management of patients with CLL. Multiple therapeutic combinations have been developed and validated for use and are summarized next.

### Fludarabine and Rituximab

The impact of sequential or concurrent administration of FR was assessed in the Cancer and Leukemia Group B (CALGB) 9712 randomized study of 104 previously untreated CLL patients.<sup>253</sup> Fludarabine was given at 25 mg/m<sup>2</sup> days 1 to 5 every 4 weeks for six cycles, with or without concurrent rituximab 375 mg/m<sup>2</sup> on day 1 of each cycle, and an additional dose on day 4 of cycle 1. Patients in both arms received rituximab 375 mg/m<sup>2</sup> weekly for four doses beginning 2 months after completion of fludarabine; thus, patients in the concurrent arm received 11 total doses of rituximab, compared to four in the sequential arm. ORR was 90 percent versus 77 percent and CR rates were 47 percent versus 28 percent in the concurrent versus sequential arm, respectively. A retrospective comparison to a fludarabine-only treatment (CALGB 9011) demonstrated improved PFS and OS with the use of FR.<sup>254</sup> This regimen had limited efficacy in patients with del 17p and del 11q. The median PFS was 42 months, and 27 percent were progression free at



5 years. Notably, there were no cases of treatment-related myeloid neoplasms occurring before disease relapse.<sup>255</sup>

### **Fludarabine, Cyclophosphamide, and Rituximab**

Fludarabine, cyclophosphamide, and rituximab (FCR) chemotherapy has been studied extensively in patients with previously treated and untreated CLL.<sup>256–259</sup> Fludarabine was administered at 25 mg/m<sup>2</sup>, cyclophosphamide at 250 mg/m<sup>2</sup> on days 2 to 4 of cycle 1 and on days 1 to 3 of cycles 2 to 6, and rituximab at 375 mg/m<sup>2</sup> on day 1 of cycle 1 and 500 mg/m<sup>2</sup> on day 1 of cycles 2 to 6. In the initial single-institution, phase II experience of 300 untreated CLL patients, this combination resulted in an exciting ORR of 95 percent with a CR rate of 72 percent and median time to progression of 80 months in patients with untreated CLL.<sup>259</sup> However, despite a younger patient population with a median age of 57 years, FCR resulted in significant and sustained myelosuppression, with 35 percent of patients experiencing cytopenias 3 months after completing therapy.<sup>260</sup> Notably, therapy-related myeloid neoplasms/myelodysplastic syndromes occurred in 5.1 percent of patients and Richter transformation in 9 percent.<sup>261</sup> FCR was subsequently compared to FC in a large multicenter trial of 817 young patients (median age: 61 years) with previously untreated CLL.<sup>262</sup> The responses observed with FCR were more modest in larger phase III trials. Patients treated with FCR had a significantly higher ORR of 90 percent with a CR rate of 44 percent as compared to FC, which resulted in an ORR of 80 percent and a CR rate of 22 percent. More important, this study established the improvement in OS with the addition of rituximab to chemotherapy. As a result, rituximab was approved for the treatment of patients with CLL in combination with chemotherapy in 2010. The use of FCR was also associated with a significantly higher risk of cytopenias that did not result in a higher incidence of serious infectious complications which were similar in both arms as was the treatment-related mortality. All genomic groups appear to benefit from the addition of FCR, except for those without any common interphase cytogenetic abnormalities and those with del 17p.<sup>263</sup> Long-term followup of FCR by both the MD Anderson group and the German CLL Study Group suggests that the patients benefiting most from FCR may be those with IGHV mutated disease where prolonged complete remissions can be obtained. For patients with IGHV unmutated disease, there does not appear to be any evidence of sustained stable remission, with all eventually relapsing and requiring additional therapy.<sup>263,264</sup>

Given the poor tolerability and increased toxicity observed with the use of FCR in elderly patients and patients with compromised renal function and to enhance efficacy of FCR, multiple variations of the regimen were tested with similar results in small cohorts of patients.<sup>265,266</sup> None of these regimens are commonly used, and the advent of alternative agents have made these regimens of limited utility.

### **Bendamustine and Rituximab**

The combination of bendamustine and rituximab (BR) has been tested both in patients with relapsed CLL and in patients with untreated CLL. In the first BR study with relapsed CLL, bendamustine was administered to 78 patients at a dose of 70 mg/m<sup>2</sup> on days 1 and 2, with rituximab 375 mg/m<sup>2</sup> on day 0 of cycle 1 and 500 mg/m<sup>2</sup> on day 1 of cycles 2 to 6. The ORR was 59 percent with a CR rate of 9 percent and a median PFS of 14.7 months. Minimal activity was observed in patients with del 17p.<sup>267</sup> In the subsequent followup study, 117 patients with previously untreated CLL were treated at the same schedule but with a higher dose of bendamustine at 90 mg/m<sup>2</sup> on days 1 and 2 and resulted in an ORR of 91 percent with a CR rate of 33 percent.<sup>268</sup> In the large, multicenter, CLL10 trial, 564 patients with previously untreated CLL, good performance status, and low comorbidity burden were randomized to FCR or BR.<sup>269</sup> ORR was similar in both arms at 97 percent, but CR rates were

higher in the FCR arm at 40 percent versus 31 percent with BR. MRD rates were also higher with FCR (74 percent vs. 62 percent). Median PFS was 53 months in the FCR arm and 43 months in the BR arm. FCR was more toxic and severe neutropenia was more often observed in the FCR arm (87 percent vs. 67 percent), as were severe infections (39 percent vs. 25 percent). Treatment-related mortality was 3.9 percent with FCR and 2.1 percent with BR. Given these results, FCR appears to be the more effective therapeutic option for younger patients and patients without significant comorbid conditions, and BR can be used for older and unfit patients.

### **Other Chemoimmunotherapeutic Regimens**

Pentostatin and rituximab and pentostatin, cyclophosphamide, and rituximab (PCR) both resulted in promising ORR (91 percent vs. 76 percent) and CR rates (41 percent vs. 27 percent).<sup>237,270</sup> Importantly, the PCR regimen appeared to be generally well tolerated in young and older patients and in patients with high-risk genetic features. Levels of Mcl-1, an antiapoptotic protein, were also shown to be predictive of response and OS.<sup>34</sup> The most common toxicities were neutropenia and thrombocytopenia. Similarly, cladribine and cladribine plus cyclophosphamide were also combined with rituximab and resulted in predictable efficacy and toxicity.<sup>271–273</sup>

## **KINASE INHIBITORS**

The introduction of BCR kinase inhibitors has significantly improved the management options for patients with CLL and represents a group of agents that very likely will change the natural history of this disease. Early results with these agents, including ibrutinib and idelalisib that target BTK and PI3K, respectively, have shown promising efficacy and excellent tolerability, but long-term data are limited to 4 years of followup; decision rules need to be developed that allow for eventual treatment discontinuation.<sup>72</sup> Much of what was learned from the transition of chemoimmunotherapy management of chronic myeloid leukemia will likely become relevant to CLL as additional followup develops with the use of BCR antagonists in this disease.

### **Ibrutinib**

Ibrutinib is a first-in-class, irreversible inhibitor of BTK and covalently binds to Cys-481 near the ATP-binding domain of the BTK molecule and abrogates enzyme activity and BCR-mediated survival signals. Ibrutinib also has the ability to irreversibly target ITK in T cells and other Tec family of kinases.<sup>274</sup>

A 420-mg daily oral dose of ibrutinib has been used in the treatment of patients with relapsed CLL and demonstrated an ORR of 71 percent with an additional 20 percent of patients experiencing partial response (PR) with lymphocytosis (PR+L), which, interestingly, does not appear to predict for inferior PFS.<sup>275</sup> The responses observed with ibrutinib are sustained and resulted in PFS of 75 percent at 26 months.<sup>92</sup> Patients with del 17p had an ORR of 55.9 percent with a median duration of response of 25 months.<sup>276</sup> Historical comparisons reveal a significantly improved response rate and PFS when ibrutinib was compared to either cyclin-dependent kinase (CDK) inhibitors or other conventional therapies used in the past for patients with del 17p.<sup>91</sup> Similar exciting results were reported in elderly, treatment-naïve patients treated with ibrutinib with ORR of 71 percent and 13 percent PR+L. These responses also appear to be sustained over time with PFS of 96.1 percent at 2 years and 92 percent at an extended followup of 5 years.<sup>277</sup> Ibrutinib in general is well tolerated, with the most common side effects being mild diarrhea, nausea, and fatigue. A randomized study comparing ibrutinib to ofatumumab in relapsed CLL confirmed the benefits of ibrutinib with improved response rate, PFS, and OS.<sup>278</sup> However, this

study also demonstrated a higher incidence of both minor bleeding and atrial fibrillation with ibrutinib. The bleeding diathesis observed with ibrutinib is possibly caused by a collagen-mediated platelet aggregation defect.<sup>278,279</sup> Caution should be taken to avoid the use of ibrutinib in patients on concurrent anticoagulation with warfarin, and treatment should be held 3 to 7 days before and after minor and major surgical procedures, respectively. The pathophysiologic reason for increased risk of atrial fibrillation with ibrutinib is uncertain. Ibrutinib treatment also results in a progressive decline in the incidence of infectious complications with ongoing therapy, primarily as a result of disease control.<sup>92</sup> Most patients do not require routine antimicrobial prophylaxis commonly used with chemoimmunotherapy. Moreover, improvements are also observed in stress, depressive symptoms, fatigue, and quality of life in patients treated with ibrutinib.<sup>280</sup> Based on these exciting data, ibrutinib was approved in 2014 for the treatment of all patients with relapsed CLL and for the treatment of all patients with del 17p CLL regardless of prior therapy.

Treatment with ibrutinib does not result in CRs in most patients, and discontinuing this treatment in heavily pretreated patients often results in patients experiencing rapid disease progression. Therefore, we currently recommend that ibrutinib be continued in responding patients until disease progression or unacceptable toxicity. However, this chronic exposure to kinase inhibitors might result in the emergence of resistant malignant cell clones, although this has been quite rare.<sup>72</sup> Data derived from whole-exome sequencing of paired samples at baseline and at the time of relapse while being treated with ibrutinib identified a cysteine-to-serine mutation in BTK at the binding site of ibrutinib and three distinct mutations in PLC $\gamma_2$ .<sup>281</sup> Functional analysis revealed that the C481S mutation of BTK results in a protein that is only reversibly inhibited by ibrutinib. The R665W and L845F mutations in PLC $\gamma_2$  are both potentially gain-of-function mutations that lead to autonomous BCR activity. Interestingly, these mutations were not found in any of the patients with prolonged lymphocytosis who were taking ibrutinib, suggesting an alternative and as yet unidentified mechanism for the persistence of lymphocytosis in those patients. Furthermore, the clinical impact of this persistent lymphocytosis was not demonstrated, with individuals having persistent lymphocytosis actually having similar outcome to those patients with PRs or better.

Ibrutinib is being combined with various other agents to improve the depth of response and outcomes. The combination of ibrutinib and rituximab in patients with high-risk CLL was generally well tolerated and resulted in an ORR of 95 percent and a PFS of 78 percent at 18 months.<sup>282</sup> PFS was 72 percent at 18 months in patients with del 17p. The combination of bendamustine and rituximab with ibrutinib was also compared to bendamustine and rituximab alone for patients with relapsed disease and demonstrated an improvement in PFS without any significant increase in toxicity.<sup>282a</sup> This outcome resulted in the approval of ibrutinib in that setting in combination with chemoimmunotherapy; however, the lack of an ibrutinib-only comparison group limits the utility of these data, especially for patients who are not candidates for chemoimmunotherapy and those exploring nonchemotherapy options. Multiple other trials are ongoing with ibrutinib in the frontline setting and in comparison to chemoimmunotherapy. These trials and the development of newer BTK inhibitors have the potential to change the treatment paradigms for CLL.

### Idelalisib

Idelalisib is an orally bioavailable, first-in-class isoform selective PI3K- $\delta$  inhibitor that promoted apoptosis of CLL B cells *ex vivo*, along with abrogating the survival signals provided by the microenvironment.<sup>283</sup> The PI3K family of enzymes are involved in an extraordinarily diverse

group of cellular functions, including cell growth, proliferation, differentiation, motility, survival, and intracellular trafficking.<sup>284</sup> Many of these functions relate to the ability of class I PI3Ks to activate the PI3K/AKT/mTOR (mammalian target of rapamycin) pathway.<sup>285</sup> The p110 $\delta$  isoform regulates different aspects of cellular proliferation and survival and is constitutively overexpressed in CLL B cells<sup>286</sup>; consequently, targeting it with specific inhibitors has become a useful therapeutic option.<sup>72</sup>

In a phase I trial of 54 patients with relapsed/refractory high-risk CLL, idelalisib resulted in an ORR of 72 percent (including PR+L).<sup>71</sup> The median PFS was 15.8 months. Therapy was generally well tolerated, with the most commonly observed grade 3 or greater adverse events being pneumonia (20 percent), neutropenic fever (11 percent), and diarrhea (6 percent). The combination of idelalisib and rituximab was also compared to rituximab and placebo in a phase III trial and resulted in an ORR of 81 percent versus 13 percent and PFS at 1 year in excess of 90 percent versus 5.5 months in the rituximab and placebo arms, respectively.<sup>287</sup> Serious toxicities observed with idelalisib included transaminase elevations, diarrhea with colitis, and pneumonitis, which were primarily observed after continued drug exposure. Based on these results, idelalisib was approved in 2014 in combination with rituximab for the treatment of patients with relapsed CLL with significant comorbid conditions that would make them ineligible for treatment with standard chemotherapy. Similar to ibrutinib, idelalisib has been studied in various combinations with different chemoimmunotherapy regimens, but these studies resulted in an increased risk of deaths from infectious complications, limiting further development of these combinations. Moreover, idelalisib use in patients with previously untreated CLL has been associated with life-threatening hepatotoxicity; hence, its use is not recommended in that setting.

### Venetoclax

Venetoclax (ABT-199) is an oral BCL-2–targeting agent that has shown impressive activity with deep remissions in patients with relapsed and refractory CLL, including those who have del 17p.<sup>287a</sup> Bcl-2 is an anti-apoptotic protein overexpressed in CLL and causes disruption of apoptosis in CLL cells. Early efforts with the first-generation compound navitoclax, demonstrated single-agent activity, but also resulted in profound thrombocytopenia as a result of off-target effects on Bcl-xl expressed in platelets. Venetoclax is significantly more potent and more specific to Bcl-2 with limited off-target effects.<sup>288</sup> Initial approval of venetoclax was based on a study of 107 patients with relapsed CLL with del 17p. It demonstrated an ORR of 74 percent and a 12-month PFS of 72 percent. Similar results, with equally deep remissions, were observed when venetoclax was utilized in patients with relapsed CLL regardless of del 17p status.<sup>289</sup> Venetoclax was also effective in patients who did not respond to ibrutinib therapy, a cohort of patients who have traditionally experienced extremely poor outcomes.<sup>290</sup> Therapy with venetoclax was initially complicated by extreme tumor lysis syndrome, especially in patients with a high burden of disease, which slowed clinical development of this agent. However, this risk can be mitigated by employing stepwise increases in dose with close inpatient monitoring for patients with high tumor burden. Combination studies are under way with kinase inhibitors and monoclonal antibodies to further improve outcomes.

### Lenalidomide

Lenalidomide is an immunomodulatory analogue of thalidomide and is approved for the treatment of multiple myeloma and myelodysplastic syndrome.<sup>291</sup> Lenalidomide has also shown exciting efficacy in multiple studies in patients with previously treated and untreated CLL.<sup>292–296</sup> Multiple dose levels and schedules have been tested in CLL,

with varying responses. From compilation of all the data, a 5-mg daily continuous dose of lenalidomide appears to be the best-tolerated dose in the vast majority of patients with CLL.<sup>297</sup> The responses observed with CLL are mostly PRs in 40 to 60 percent of patients, with approximately 10 percent of the patients experiencing a CR. However, response improves with ongoing therapy. Cytopenias are the most common, and dose-limiting, side effect. Patients can also experience tumor flare and tumor lysis, especially at the start of therapy. Care should be taken to minimize these reactions with the early use of steroids and aggressive hydration and uricosuric agents. In a phase III study, lenalidomide was shown to increase mortality in patients older than 80 years of age and, thus, is not recommended for the elderly. However, long-term results indicate an improvement in OS. Two studies have demonstrated an improved PFS with maintenance lenalidomide, in both untreated and relapsed patients, achieving at least a PR after chemoimmunotherapy. This resulted in the approval of lenalidomide as maintenance therapy for patients with CLL. Combination of lenalidomide with various monoclonal antibodies has also resulted in improved sustained responses, and such combinations are a promising therapeutic option.<sup>298–300</sup> Despite the advent of kinase inhibitors, lenalidomide continues to be an exciting agent for the treatment of CLL as it is one of the few agents that have the potential to reverse the immune dysfunction associated with CLL with efficacy in patients with del 17p disease.<sup>49,301–304</sup>

## CHIMERIC ANTIGEN RECEPTOR T CELLS

Chimeric antigen receptor T (CAR-T) cells are engineered autologous lentiviral modified T cells that contain an altered TCR targeting a surface antigen (e.g., CD19) on the surface of CLL B cells. The modified TCR has a higher affinity and specificity toward the target antigen and is not major histocompatibility complex (MHC) restricted. Multiple CAR-T cells have been developed and tested in clinical trials, with exciting results.<sup>305,306</sup> These modified T cells persisted *in vivo* for an extended period and were able to induce prolonged clinical responses in the majority of patients.<sup>306a</sup> The treatment is associated with severe cytokine release syndrome and macrophage activation syndrome that require aggressive and intensive supportive care and anti-IL-6-directed therapy. These modified CAR-T cells also result in the sustained elimination of normal B cells and subsequent sustained hypogammaglobulinemia that require ongoing supportive care and infection prophylaxis to limit the incidence of infectious complications in these patients.<sup>306</sup> Development of these agents on a larger scale is ongoing to allow for larger studies to confirm their promising effect.

## OTHER AGENTS

Blinatumomab is an engineered bispecific T-cell engaging (BiTE) antibody that has shown promising activity in CD19+ B-cell malignancies, including CLL and ALL.<sup>248</sup> It was approved in 2014 for the treatment of relapsed ALL. Blinatumomab engages the CD19+ cell and brings it in close proximity to a CD3+ T cell that is also activated upon binding to the bispecific antibody. The resultant interaction leads to the apoptosis of the CD19+ B cells. The antibody has shown promising and durable responses and is currently in clinical trials for CLL. Therapy is complicated by hypogammaglobulinemia, cytokine release, and neurologic symptoms.<sup>307</sup>

CDK inhibitors like flavopiridol and dinaciclib have also demonstrated promising activity in patients with CLL and are able to induce durable remissions even in patients with high-risk del 17p disease with or without concomitant chemotherapy.<sup>308–311</sup> Therapy with CDK

inhibitors is complicated by fulminant hyperacute tumor lysis that may require aggressive supportive care, including hemodialysis. Diarrhea and fatigue are also seen as off-target effects.

## HEMATOPOIETIC CELL TRANSPLANTATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

With the advent of effective therapy for patients with relapsed and refractory disease and even for patients with high-risk disease, the role of hematopoietic cell transplantation (HCT) for the routine management of patients with CLL is diminishing. Autologous transplantation has the ability to induce deeper and more frequent remissions that may result in longer disease-free remission but does not translate into an OS advantage and is associated with significantly higher morbidity and mortality and secondary cancers, including therapy-related myeloid neoplasms that may impact long-term survival.<sup>312–314</sup> Moreover, autologous transplantation, which is essentially high-dose chemotherapy, is unable to overcome the chemoresistance conferred by del 17p or TP53 mutation. Consequently, autologous transplantation has no role in the routine management of patients with CLL.

Allogeneic HCT potentially offers a curative approach for patients with CLL, but its utility and timing are currently under considerable debate given the availability of multiple novel agents. Myeloablative conditioning prior to allogeneic HCT is associated with an unacceptably high transplant-related mortality (TRM) in excess of 30 percent and is not generally recommended.<sup>314–316</sup> Nonmyeloablative conditioning regimens afford the opportunity to reduce TRM without impacting the graft-versus-leukemia (GVL) effect. Long-term disease-free survival is generally in the 30 to 45 percent range. However, nonrelapse mortality (NRM) is still in the 15 to 30 percent range, but decreasing with improvements in supportive care techniques. Moreover, chronic graft-versus-host disease still impacts long-term quality of life after nonmyeloablative allogeneic HCT in approximately 25 percent of survivors.<sup>317–320</sup> Various patient-, disease-, and transplant-specific factors at the time of transplantation impact HCT outcomes.<sup>321</sup> In particular, the number of prior treatments and the presence of a complex karyotype contribute to success and also toxicity observed after transplantation.<sup>322–324</sup> Studies have identified that implementation of an effective quality management system and experience of the transplantation center are associated with a significant decrease in NRM.<sup>325–327</sup> Patients who relapse after HCT can sometimes be effectively salvaged with the use of currently approved novel therapeutic agents. Given the availability of novel therapeutics that include BTK, PI3K, and BCL-2 inhibitors, patients should be offered therapy with these novel agents. Consideration of nonmyeloablative HCT should be reserved for those who do not respond to these agents. Moreover, patients who progress after treatment with kinase inhibitors should be referred to a treatment center with established expertise in treating CLL, as this can significantly impact their therapeutic options and outcomes.<sup>325–327</sup> The American Society of Blood and Marrow Transplantation also recommends consideration for an allogeneic HCT for patients not responding to BCR inhibitors and for those who do not respond to BCR inhibitors while receiving Bcl-2 inhibitors.<sup>327a</sup>

## SPLENECTOMY

Splenectomy is rarely used today given the availability of effective therapeutic agents to treat CLL. It can be an effective option for ameliorating refractory autoimmune hemolytic anemia and thrombocytopenia for which medical management has been unsuccessful.<sup>328</sup> It can also be used to provide relief to patients with symptomatic splenomegaly secondary to refractory disease.<sup>329–331</sup>



## RADIATION

Involved field radiation therapy is an extremely useful treatment modality for the management of locally symptomatic lymphadenopathy and isolated Richter transformation. Splenic irradiation can be used in patients with symptomatic splenomegaly, but its efficacy is limited and short-lived, with significant and sometimes prolonged myelosuppression.<sup>332–334</sup> Systemic radiation or extracorporeal photopheresis has no role in the routine management of patients with CLL.<sup>335–337</sup>

## LEUKAPHERESIS

Patients with CLL will very rarely experience hyperviscosity-related signs and symptoms secondary to hyperleukocytosis. However, this modality can be used for the occasional patient who becomes symptomatic from hyperleukocytosis. When this is initiated, it should be performed in conjunction with conventional therapy to cytoreducate the CLL. Leukapheresis has been used as a therapeutic option in the past for patients with refractory disease, but it results in modest and transient benefits.<sup>338–340</sup> With the advent of modern therapeutic options, the role of leukapheresis is limited in the management of routine CLL patients with hyperleukocytosis. While the BCR signaling agents can cause profound hyperleukocytosis in some patients, it virtually never results in symptoms mandating leukapheresis.

## ASSESSMENT OF RESPONSE

Patients who complete therapy should be assessed for response. This involves performing a detailed history for any disease-related symptoms, physical examination for any palpable lymphadenopathy, and laboratory studies to evaluate for persistent cytopenias or leukocytosis. Outside of a clinical trial, if the patient fails to have a CR on routine history, physical evaluation, and blood counts, then a CT scan and a marrow biopsy have limited utility. However, if the patient does achieve a CR on clinical and laboratory evaluation, we recommend proceeding with a CT scan with contrast of the chest, abdomen, and pelvis, along with a marrow aspirate and biopsy in the event that the CT scan is negative. These patients should undergo evaluation for MRD on the marrow aspirate. Formal response criteria for disease evaluation were revised with the advent of kinase inhibitors. Conventionally, patients with no evidence of disease on CT scan and marrow biopsy with complete hematologic count recovery would be classified as having a complete remission. Patients with a CR but with persistent cytopenias that are related to drug toxicity are classified as having CR with incomplete count recovery (CRi). These are also detailed in the IWCLL-2008 criteria.<sup>1</sup> Evaluation of the marrow aspirate and biopsy is also useful in assessing the etiology of cytopenias that can be seen frequently after chemotherapy use. We recommend waiting until count recovery or 3 to 6 months after chemotherapy to repeat a marrow biopsy for persistent cytopenias. Occasionally, patients will have no evidence of disease on flow cytometry of the aspirate or on the biopsy specimen but may have nodular lymphoid aggregates. If these aggregates are confirmed to be consistent with a collection of CLL cells by immunohistochemical studies, then these patients should be classified as having a nodular PR. Given the advent of kinase inhibitors, the response criteria are undergoing significant modifications, especially because most patients will not experience traditional responses as observed with chemotherapy use despite having similar or even better outcomes.<sup>341</sup> This is especially evident in patients with PR+L after kinase inhibitor therapy with ibrutinib who demonstrate significant and sustained improvement in clinical signs and symptoms related to CLL and in lymphadenopathy and organomegaly and potentially may have a longer progression-free interval as compared to people with a more conventional partial remission.<sup>275</sup>

## EVALUATION FOR MINIMAL RESIDUAL DISEASE

CLL is currently considered incurable, but a subset of patients who achieve a complete remission will have undetectable disease even after using highly sensitive techniques. The evaluation for MRD is now fairly well established and can be performed by either flow cytometry or allele-specific oligonucleotide polymerase chain reaction–based methods.<sup>342–345</sup> Both these tests have excellent comparable sensitivity and are able to identify one leukemia cell in 10,000 leukocytes.<sup>343</sup> These tests can be performed on blood, but sensitivity is higher when performed on marrow aspirate, especially with the current use of monoclonal antibodies that are able to effectively clear the circulating blood of the disease but are not equally effective in eliminating disease from the marrow or lymph nodes. Patients with MRD-negative disease at the completion of therapy experience longer treatment-free and OS periods.<sup>346,347</sup> This advantage in outcomes has been shown both for chemotherapy-based regimens and for regimens based primarily on antibodies.<sup>262,347</sup> The utility of MRD assessment in the era of kinase inhibitors is currently under discussion as kinase inhibitors are not typically able to induce deep remissions as monotherapy in the majority of patients with short followup. MRD assessment, however, can be used as a surrogate for survival outcomes and to potentially develop a stopping rule for kinase inhibitor–based combination approaches.

## RECOMMENDATIONS FOR TREATMENT OF PATIENTS WITH UNTREATED DISEASE

All patients should be offered therapy on a clinical trial whenever possible. This is increasingly important as therapy for CLL is evolving rapidly, and clinical trials are required to answer the multiple questions regarding the best possible therapeutic option. For patients being treated off trial, we recommend discussion of the potential risks and benefits of chemoimmunotherapy with FCR, particularly when low-risk disease is present (e.g., IGHV mutated disease) that might result in prolonged remission. For those with IGHV unmutated disease, chemoimmunotherapy with FCR or alternative non-chemotherapy-based regimens are considered. For patients older than age 65 years and those with multiple comorbid conditions or renal insufficiency, we recommend therapy with non-chemotherapy-based regimens, including antibodies and/or kinase inhibitors. Patients with del 17p should be treated with ibrutinib in the first-line setting.

## RECOMMENDATIONS FOR TREATMENT OF PATIENTS WITH RELAPSED DISEASE

Patients who relapse after conventional chemoimmunotherapy-based approaches should, in most cases, be treated with ibrutinib unless a need for chronic anticoagulation with warfarin (Coumadin) is required. In such settings, treatment with idelalisib with or without rituximab should be considered. With both ibrutinib and idelalisib, the benefit of adding rituximab at this time is not known. Repeating a different chemoimmunotherapy regimen is also an option in younger and otherwise healthy patients but is associated with higher toxicity and risk of secondary neoplasms. Patients who relapse after one kinase inhibitor should be treated with the other and referred to a specialized center for clinical trial participation on subsequent relapses. Venetoclax is currently approved for patients with relapsed disease and del 17p but has also been shown to have significant efficacy in patients relapsing after kinase inhibitors.

## ● SECONDARY CANCER RISK WITH CHRONIC LYMPHOCYTIC LEUKEMIA

Patients with CLL have a significantly higher risk of developing a secondary malignancy. This includes cancers of the skin, connective tissue and peripheral nerves, eye, lip and oral cavity, lung, kidney, colorectal, prostate, breast, and genitourinary system.<sup>348</sup> Skin cancer is one of the most common cancers seen in patients with CLL.<sup>349–351</sup> This includes basal cell carcinoma, squamous cell carcinoma, cutaneous melanoma, and Merkel cell carcinoma. Patients with skin cancers also appear to have a worse prognosis from their melanoma and Merkel cell cancer if they also have a concomitant CLL history.<sup>348,352</sup> This is also true for solid tumors, as patients with breast, colorectal, prostate, lung, and kidney cancers have an inferior prognosis if they have a preexisting diagnosis of CLL.<sup>353</sup> We therefore recommend an annual mammogram and Papanicolaou test for female patients, annual prostate evaluation in male patients, and annual dermatologic evaluation and screening colonoscopy every 5 years for all patients with CLL. Multiple myeloma also occurs at a higher frequency in patients with CLL but appears to arise from a separate B-cell clone.<sup>354–356</sup> Non-Hodgkin lymphomas are typically not associated with CLL, and appearance of a large cell lymphoma is considered to be Richter transformation.<sup>357</sup>

## ● MANAGEMENT OF INFECTIOUS COMPLICATIONS

Infectious complications constitute the leading cause of morbidity and mortality in patients with CLL. Patients with CLL develop progressive hypogammaglobulinemia with progressive disease that results in a higher incidence of infections with encapsulated organisms like *Streptococcus pneumoniae* and *Haemophilus influenzae*.<sup>358</sup> This has historically been further compounded by the effect of cytotoxic chemotherapeutics like fludarabine or antibodies like alemtuzumab that cause profound T-cell depletion along with worsening normal B-cell depletion and resultant hypogammaglobulinemia.<sup>359</sup> This combined effect of disease and therapy results in a higher incidence of opportunistic bacterial and viral infections with herpes simplex virus, CMV, herpes zoster virus, and *Listeria monocytogenes*. Fungal infections with *Cryptococcus neoformans* and *Pneumocystis jiroveci* are also seen in these patients and patients treated with high-dose glucocorticoids.<sup>359–361</sup> Patients respond to appropriate antibiotics early in their disease course but might require prolonged and repeated courses of antibiotics later in their disease course and after therapy with chemotherapeutic agents. Prophylaxis with acyclovir for herpes zoster infections and with trimethoprim-sulfamethoxazole for *P. jiroveci* is routinely used for patients with prior therapy with nucleoside analogues.<sup>362</sup> Fungal prophylaxis is also employed with voriconazole or posaconazole for the prevention of invasive aspergillosis in patients on high-dose steroids. Patients treated with ibrutinib experience a lower incidence of infection, possibly because of effective disease control and despite a significant improvement in Ig levels.<sup>92</sup>

Hypogammaglobulinemia is universally present in patients with CLL and progressively worsens with advancing stage of the disease. Intravenous immunoglobulin (IVIG) at doses of 250 to 600 mg/kg administered every 4 to 6 weeks may result in a significant reduction of major infections requiring intensive supportive care and a modest reduction in the incidence of clinically significant infections. However, there is no significant improvement in survival.<sup>363–365</sup> Consequently, we recommend the judicious use of IVIG in patients at high risk of developing infectious complications.

Along with the disease-related immune defects, patients with CLL also respond poorly to routine prophylactic vaccinations. Response to protein-conjugated vaccines can be modestly augmented with concomitant use of antihistamine (H<sub>2</sub>) blockers.<sup>366–369</sup> Protein-conjugated vaccines also appear to be more immunogenic than polysaccharide vaccines. In the absence of definitive data on the use of vaccines in CLL, we recommend following the 2014 adult immunization schedule for immunocompromised adults, recommended by the Advisory Committee on Immunization Practices. This includes annual influenza vaccine and pneumococcal 13-valent conjugate (PCV-13) vaccine.<sup>370–372</sup> Vaccination with live virus vaccines, including the zoster, varicella, and measles, mumps, and rubella (MMR) vaccines, is contraindicated in patients with CLL.<sup>372</sup>

The routine use of granulocyte colony-stimulating factor (G-CSF) for patients with CLL is not recommended. G-CSF and granulocyte-macrophage colony-stimulating factor (GM-CSF) can be used to treat therapy-related neutropenia and patients with febrile neutropenia to shorten the duration and severity of illness.

## ● MANAGEMENT OF AUTOIMMUNE COMPLICATIONS OF CHRONIC LYMPHOCYTIC LEUKEMIA

Patients with CLL have a greater risk of developing autoimmune complications, including autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), and pure red cell aplasia (PRCA). In the majority of patients, the nonmalignant B-cell clone produces the auto-antibody, reflecting a dysregulation of humoral immune tolerance.<sup>373,374</sup> Patients who present with autoimmune cytopenias may not have a worse prognosis than patients in whom cytopenias develop because of extensive marrow infiltration by the disease.<sup>328,375–377</sup> AIHA can present in up to a third of patients with CLL at some time during the course of their disease and in a small proportion of patients (10 to 15 percent) at the time of diagnosis.<sup>328</sup> Patients present with signs and symptoms of acute onset of anemia with weakness, fatigue, lethargy, and shortness of breath on exertion. Physical examination reveals pallor, jaundice, lymphadenopathy, and hepatosplenomegaly. Laboratory evaluation reveals anemia, elevated LDH, hyperbilirubinemia, positive direct Coombs test, and decreased serum haptoglobin. Not all patients who have a positive Coombs test, however, develop hemolysis. Most patients have warm reactive antibodies, but some patients may develop cold agglutinin disease.<sup>373</sup> Occasionally, patients may develop concomitant ITP with AIHA or Evans syndrome. Certain miRNAs have been correlated with the development of AIHA, but their mechanistic explanation in the pathogenesis of AIHA is lacking.<sup>378</sup>

ITP results in development of sudden profound thrombocytopenia with associated bleeding diathesis. Accurate diagnosis requires a marrow aspirate and biopsy to evaluate the extent of CLL in the marrow and the presence of adequate or increased numbers of megakaryocytes.

Patients often require periodic red cell transfusions for symptomatic anemia, and platelet transfusions may be employed in patients with severe thrombocytopenias with bleeding complications. Glucocorticoids have been the mainstay of treatment for AIHA and ITP. However, steroids need to be dosed at 0.5 to 1.0 mg/kg/day for 2 to 3 weeks, followed by a slow taper over several weeks. Unfortunately, a large number of patients relapse after discontinuation of the steroids and may need subsequent therapy with IVIG or rituximab. The early use of IVIG and weekly rituximab for four doses may allow for better disease control and rapid glucocorticoid withdrawal. Other immunosuppressive agents that have been used include cyclosporine, the dose of which can be titrated to stable hemoglobin or platelet levels or serum

trough levels between 100 and 150 ng/mL.<sup>331</sup> Erythropoiesis-stimulating agents have been used and may decrease the frequency of packed red cell transfusions; however, their use can be complicated by polycythemia and thromboembolic disease.<sup>379,380</sup> Thrombopoietin agonists can also be considered in refractory patients, but conclusive data are currently lacking.<sup>381</sup> Refractory cases may require definitive treatment with therapeutic agents for the underlying CLL. Initiating nucleoside analogue-based chemotherapy in patients with preexisting AIHA or ITP is not contraindicated, but use is contraindicated if these complications arise while patients are on these agents. In general for patients presenting with both active CLL and autoimmune manifestations, our approach is to first treat the autoimmune complication and then the CLL if symptoms persist. However, ibrutinib treatment for CLL is associated with a low rate of treatment-emergent autoimmune cytopenias, and patients with existing cytopenias who were treated with ibrutinib were able to discontinue their cytopenia directed treatment.<sup>381a</sup> Therefore, there might be a role for ibrutinib in the treatment of patients who fail to respond to conventional treatments for autoimmune cytopenias.

PRCA is a rare complication of CLL that can arise spontaneously. PRCA needs to be differentiated from other forms of anemia, including AIHA and anemia resulting from extensive marrow infiltration. PRCA is characterized by a decrease in red cell precursors.<sup>382</sup> Additionally, select infections, such as parvovirus, can cause PRCA in CLL. The etiology of disease-related PRCA is believed to be related to the cytotoxic effect of a T-cell large granular lymphoma (T-LGL) clone on erythroid progenitor cells (Chap. 5). These CD3-, CD8-, and CD57-positive cells slowly accumulate in the marrow of patients with PRCA. Occasional cases have been observed after parvovirus B19 infection.<sup>383</sup> Treatment with immunosuppressive agents like glucocorticoids, cyclophosphamide, and/or cyclosporine results in reticulocytosis and slow correction of the anemia over several weeks.<sup>384</sup> IVIG, rituximab, and antithymocyte globulin have also been used in refractory cases.<sup>385–388</sup>

## ● RICHTER SYNDROME

Richter syndrome (RS) is defined as a transformation of CLL into an aggressive, high-grade, large B-cell non-Hodgkin lymphoma. The large cell lymphoma is typically of the activated B-cell (ABC) subtype by immunohistochemistry.<sup>389</sup> Incidence estimates vary from 2 to 10 percent.<sup>390</sup> Transformation typically occurs in patients with unmutated IGHV genes, but occasionally may be seen in patients with mutated IGHV genes who have been heavily pretreated.<sup>391</sup> The development of transformation may be related to the evolution of an alternate clone of malignant B cells distinct from the original CLL clone in a subset of patients.<sup>392</sup> Transformation appears to occur independent of disease stage, duration of disease, type of therapy, or response to therapy. However, the presence of bulky and advanced-stage disease; IGHV unmutated disease, especially with involvement of 4 to 39 loci; high CD38 expression; absence of del 13q; and presence of del 17p or del 2p and trisomy 12, typically in combination with NOTCH1 mutations, may predict the development of RS.<sup>393–396</sup> The syndrome is characterized by rapid development of B symptoms and rapidly progressive lymphadenopathy. Cytopenias may worsen rapidly because of transformation in the marrow. LDH is also increased in the majority of patients. Patients typically have complex karyotype and involvement of TP53, ATM, RB (retinoblastoma), and c-myc genes.<sup>397</sup> PET scans have generally no role in the routine management of patients with CLL but can be very useful in the diagnosis of transformation. A standardized uptake value (SUV) of 5 or less on a lymph node by PET scan has a high negative predictive value, and patients with a higher SUV require an excisional biopsy for definitive diagnosis.<sup>156,398,399</sup> Prognosis of RS is related to the clonality of the malignant clone in that patients with clones unrelated

to the CLL tend to have a much better prognosis than patients with a clonally related RS.<sup>389</sup> Additionally, patients who present with RS prior to treatment for their CLL often have a better outcome. Treatment of patients with RS has historically been with regimens similar to those used for the treatment of patients with large cell lymphoma, with mixed results. These include R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) or R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) and R-hyperCVXD (rituximab, fractionated cyclophosphamide, vincristine, liposomal daunorubicin, and dexamethasone). However, the use of hyperCVXD is associated with unacceptably high treatment-related mortality, and its use is not recommended.<sup>400,401</sup> The OFAR combination also has modest but short-lived responses with significant toxicities.<sup>400</sup> There is a role for ibrutinib in combination with PD-1 blocking checkpoint inhibitors for the treatment of RS. In the absence of results from clinical trials, dose-adjusted R-EPOCH, with or without BCR inhibitors, is a preferred regimen to debulk patients before proceeding with more definitive therapy, including allogeneic HCT or CAR-T cell therapy.

Along with large B-cell transformation, patients can also experience polymorphocytic leukemia (PLL) transformation, characterized by the presence of more than 55 percent polymorphocytes in the marrow, or Hodgkin lymphoma.<sup>402–404</sup> Unlike large cell transformation, patients with Hodgkin lymphoma have a similar outcome to *de novo* disease when matched by stage and can be treated with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD)-based therapy (Chap. 8).<sup>405</sup>

The polymorphocytic transformation is similar to the distinct entity of B-cell PLL, which is characterized by subacute accumulation of polymorphocytes in a predominantly older, male population with presence of chromosome 14q abnormalities and del 17p.<sup>406–408</sup> Gene-expression profiling suggests subgroups of patients with PLLs that are similar to CLL and others that are similar to mantle cell lymphoma.<sup>409</sup> Patients present with B symptoms, massive splenomegaly, and palpable lymphadenopathy. Occasionally, patients may have organ infiltration and extralymphatic invasion, including neurologic involvement and leukostasis from hyperleukocytosis.<sup>410,411</sup> Polymorphocytes have variable expression of CD5 and are negative for CD23. Indications and therapeutic options for patients with PLL are similar to those used for CLL.<sup>412–414</sup>

T-cell polymorphocytic lymphoma (T-PLL) is a distinct clinical entity that was previously classified as T-CLL. These patients typically present with B symptoms and paraneoplastic phenomenon associated with rapid splenomegaly and lymphadenopathy with leukocytosis. Evaluation results in the demonstration of polymorphocytes on tissue biopsy with markers typical for T-cell disorders, including CD3, CD5, CD4, CD7, and CD8. TCR gene rearrangements are also observed. These features help distinguish them from B-cell CLL. The most effective treatment for this entity is alemtuzumab, although regimens for aggressive T-cell lymphomas have also been used.<sup>412,415–419</sup> In general, all patients relapse from these therapies, making consolidation allogeneic transplantation a viable consideration for these patients if a donor is available and patient comorbid features allow.

## ● MONOCLONAL B-CELL LYMPHOCYTOSIS

Monoclonal B-cell lymphocytosis (MBL) can be characterized as a precursor state of CLL. Patients who have less than 5000 B-lymphocytes/ $\mu$ L in their blood, in the absence of B symptoms, lymphadenopathy, organomegaly, or cytopenias, are classified as having monoclonal B-cell lymphocytosis. Presence of a lymphoid aggregate or infiltration of lymph node or marrow by cells with a characteristic CLL phenotype establishes the diagnosis of CLL regardless of blood lymphocytosis.



The prevalence of MBL varies from 3.5 percent in normal healthy people<sup>420</sup> with normal counts to 13.5 percent to 17 percent in normal healthy people with a family history of CLL,<sup>16,421</sup> which suggests a familial predisposition to the development of CLL. The vast majority of patients with MBL have clonal populations with a characteristic CLL phenotype on sensitive flow cytometry assay that is positive for CD5 and CD23, negative for CD10, and dim positive for CD20. Other phenotypes that have been observed are the atypical CLL phenotype with positive CD5, CD23, and bright CD20 and the non-CLL phenotype that is negative for CD5 and CD23 and appears to be more consistent with low-grade lymphoproliferative disorders like marginal zone lymphoma or lymphoplasmacytic lymphoma.<sup>422</sup> MBL is more prevalent in men and increases in incidence with age. The cumulative annual risk of patients with MBL who require CLL-specific therapy is approximately 1 percent.<sup>16,17,420</sup> Retrospective analysis of samples collected on longitudinal, prospective studies have also demonstrated that the development of overt CLL is almost always preceded by MBL.<sup>423</sup> Management of patients with MBL is similar to patients with early-stage CLL, and the vast majority of these patients can be followed with watchful waiting. Like early-stage CLL, these patients are predisposed to a higher frequency of infections and also secondary malignancies, mandating followup similar to that recommended for CLL. Our own practice is to see MBL patients yearly with exams and complete blood count assessment.

## ● SMALL LYMPHOCYTIC LYMPHOMA

Small lymphocytic B-cell lymphoma (SLL) is closely related to CLL and mimics it with regard to clinical course and outcomes. The malignant B cells have similar immunophenotypic features and prognostic markers on lymph node assessment but without the presence of significant malignant monoclonal B-cell lymphocytosis on blood or marrow assessment. Treatment and outcomes of these patients are similar to those for patients with CLL, and as such this disorder should be considered analogous to CLL.<sup>1,45,424,425</sup>

## ● DIFFERENTIAL DIAGNOSES

Multiple low-grade lymphoid malignancies can mimic the presentation and laboratory evidence of CLL and are discussed in detail in the chapters on non-Hodgkin lymphomas (Chaps. 6, 7, 10–12). The most common entities mimicking CLL are briefly discussed next.

## MANTLE CELL LYMPHOMA

Mantle cell lymphoma frequently mimics the clinical presentation and laboratory findings observed in patients with CLL (Chap. 11). However, the malignant lymphocytes observed in mantle cell lymphoma express CD5 without coexpression of CD23 and have a higher level of expression of CD79. Cases of CD23-negative CLL and CD23-positive mantle cell lymphoma have been described. An important discriminating feature between the two disorders is the presence of t(11;14) in mantle cell lymphoma, which results in the bcl-1-IGH translocation and the overexpression of cyclin D1. Therapy for mantle cell lymphoma employs some of the agents used for CLL but is covered in detail elsewhere.<sup>426–429</sup>

## HAIRY CELL LEUKEMIA

Hairy cell leukemia (HCL) presents with B symptoms, splenomegaly, and the presence of typical villous “hairy” projections from the surface of its cells (Chap. 4). The cells are also strongly positive for tartrate-resistant acid phosphatase (TRAP) and express CD11c, CD25, and CD103. With the discovery of the BRAF<sup>V600E</sup> in HCL, significant improvements have

been made in the availability of therapeutic agents for the management of these patients.<sup>430–433</sup>

## OTHER LYMPHOID MALIGNANCIES

Various low-grade lymphoid malignancies can mimic the clinical and laboratory presentation of CLL and include marginal zone lymphomas and lymphoplasmacytic lymphomas. However, pathologic evaluation results in the identification of surface markers CD19 and CD20 without coexpression of CD5 or CD23. Therapy is directed at the specific lymphoid malignancy identified.<sup>434–436</sup> B-cell ALL is also included in the list of differential diagnoses, but it is an aggressive malignancy involving immature B cells with distinct molecular features and outcomes (Chap. 2). Transformation of CLL into ALL has been reported and managed with regimens suitable for ALL.<sup>437–439</sup>

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## CHAPTER 4

# HAIRY CELL LEUKEMIA

Michael R. Grever and Gerard Lozanski

### SUMMARY

Hairy cell leukemia (HCL) is an uncommon form of adult chronic B-cell leukemia. Whereas the cell of origin is uncertain, at diagnosis the characteristic leukemic cells are found in the marrow, the blood, and the spleen. Patients present with fatigue and infections, and many have splenomegaly. They are often pancytopenic, or may have isolated cytopenias, and usually have monocytopenia. The leukemic cell in classic hairy cell leukemia (HCL-c) has a characteristic immunophenotypic profile (CD11c+, CD19+, CD20+[bright], CD22+, CD25+, CD103+, and CD123+ and CD27—). A variant of hairy cell leukemia (HCL-v), which occurs less frequently, has been identified as a separate entity. A genetic mutation, *BRAF V600E*, has been identified in the majority of patients with the classic form of this disease but is not present in the variant. This mutation is also present in the hematopoietic stem cells of patients with HCL-c.

HCL is characterized by impaired marrow function and immunity, leading to a high incidence of infectious complications. Both pentostatin and cladribine are effective in achieving durable complete remissions. Long-term studies demonstrate prolonged survival of patients, but the disease-free survival curves do not plateau, suggesting that the disease is not cured but subject to relapse. Survival has been markedly improved with the introduction of purine nucleoside analogues and is estimated to be 90 percent at 5-year followup. When patients relapse, high-quality remissions can be achieved with salvage therapy.

### DEFINITION AND HISTORY

Cases of malignant diseases involving marrow that probably represented examples of hairy cell leukemia (HCL) were reported through the first half of the 20th century and designated by such terms as “lymphoid fibrosis.” They had features characteristic of HCL, including marrow replacement by mononuclear cells, marrow fibrosis, splenomegaly, and anemia and thrombocytopenia. In 1958, Bouroncle, Wiseman, and Doan described this constellation of findings in a group of patients.<sup>1</sup> At that time there was no means to characterize the immunophenotype of malignant lymphoid cells, and they called the disease “leukemic reticuloendotheliosis.” In 1966, Schrek and Donnelly described the distinctive feature of cytoplasmic projections that were evident on the blood cells in two cases of this disorder. They called these “hairy” cells.<sup>1a</sup>

**Acronyms and Abbreviations:** HCL, hairy cell leukemia; HCL-c, classic HCL; HCL-v, variant of HCL; IHC, immunohistochemical stains; IL-2, interleukin 2; MRD, minimal residual disease; SEER, Surveillance, Epidemiology, and End Results Program; SIR, standardized incidence ratio; TGF, transforming growth factor; TRAP, tartrate-resistant acid phosphatase; WHO, World Health Organization.

The designation HCL has become universally accepted as the name of this lymphocytic neoplasm, characterized by infiltration of the marrow by malignant B lymphocytes of a specific immunophenotype (see “Laboratory Features” section), often accompanied by reticular fibrosis, splenomegaly, anemia, thrombocytopenia, neutropenia, monocytopenia, and usually pancytopenia. Occasionally, there is an elevated total white cell count because of the abundance of malignant B lymphocytes in the blood. Splenectomy was the sole therapeutic approach until the early observations of responses secondary to  $\alpha$ -interferon and the purine analogues.<sup>2,3</sup> Whereas splenectomy improved the blood counts, the impact of this intervention was temporary, resulting in improvement of symptoms related to splenic sequestration. Most standard chemotherapy was ineffective and poorly tolerated. In 1984, Quesada described the benefits of daily  $\alpha$ -interferon in achieving responses in seven patients with progressive HCL.<sup>2</sup> Three patients achieved a complete response, with the remaining four having partial responses. Extensive description of the effects of  $\alpha$ -interferon on marrow showed improvement in granulopoiesis associated with increases in the number of both circulating granulocytes and platelets. While this approach was heralded as a major achievement in treating this disease, other opportunities emerged in the same time frame.

Grever and colleagues demonstrated that low-dose pentostatin was effective in achieving responses in patients with far-advanced, low-grade, B-cell malignancy.<sup>4,5</sup> Spiers reported the initial response of HCL to pentostatin in a limited number of patients,<sup>3</sup> which was followed by a larger study with a complete remission rate of 59 percent.<sup>6</sup> Kraut and colleagues subsequently showed that low-dose, less intense, intermittent pentostatin produced a higher complete remission rate (approximately 89 percent) in patients with HCL.<sup>7</sup> Others confirmed these findings,<sup>8</sup> and a large, prospective, randomized trial of pentostatin versus  $\alpha$ -interferon solidified that frontline therapy should be based on a purine analogue.<sup>9</sup> Piro and colleagues reported that cladribine produced complete responses in 11 of 12 patients with this disease.<sup>10</sup> Numerous trials with cladribine confirmed that a single course of therapy was equally capable of inducing a high percentage of long-term complete responses.<sup>11,12</sup> However, the initial trials with cladribine excluded patients with an active infection.<sup>10,11</sup> Several studies using cladribine aimed at optimizing the remission rate and attempting to reduce myelosuppression showed that either 5 to 7 days of intravenous administration or subcutaneous injection produced responses, but these alternate approaches did not consistently reduce the risks for febrile neutropenia.<sup>13–15</sup> In contrast, the reported frequency of febrile neutropenia was less in a study using intermittent administration of pentostatin<sup>9</sup> compared to those reported with cladribine.<sup>10</sup> Consequently, either purine nucleoside analogue is now used to induce remission.

In patients with an active infection, it is advisable to control the infection before initiating immunosuppressive chemotherapy. However, if this is not possible, then administration of either pentostatin alone or following  $\alpha$ -interferon may be effective in controlling the underlying disease.<sup>9,16</sup> If  $\alpha$ -interferon is initially used to improve the neutrophil count and control the leukemia, the subsequent use of pentostatin to achieve complete remission is not compromised. Therefore, some investigators have initially treated patients with HCL complicated by infection with  $\alpha$ -interferon, which was followed by pentostatin in an effort to reduce infectious complications.<sup>16</sup> While the use of filgrastim does reduce the degree and the duration of neutropenia, it does not reduce the number of febrile episodes in patients being treated with cladribine, in comparison to historical controls.<sup>17</sup> However, it may be helpful in treating serious infections in neutropenic patients with HCL who are in a precarious medical condition. The optimal management of actively infected patients with HCL requiring treatment is still challenging. Improvement in blood cell counts of patients with HCL complicated by serious infections occurs

following administration of vemurafenib. This BRAF inhibitor has reduced hematologic toxicity and may prove useful for patients with HCL with active uncontrolled infection.<sup>18</sup> The remarkable advances in diagnosis and therapeutics with purine analogues over the past 25 years have clearly changed the natural history of this disease.<sup>19</sup> Patients may now lead a near-normal life, despite the likelihood of intermittent relapses requiring re-treatment.<sup>20,21</sup> Although the risk for serious bacterial infection is greatest during initial therapy, there are some delayed risks associated with impaired recovery of T-lymphocyte cell numbers and function following administration of purine analogues.<sup>20,22</sup>

In patients who achieve a complete morphologic remission, the presence of minimal residual disease (MRD) has been repeatedly shown by immunohistochemical staining of the marrow.<sup>20</sup> Some patients in hematologic remission with evidence of MRD may live years without hematologic relapse. Studies are required to determine whether therapeutic intervention for MRD will improve patient outcomes.<sup>23,24</sup> Consequently, further research to define the optimal therapeutic approach and timing for evaluation of response is needed.

The excellent results achieved with purine nucleoside induction associated with high percentages of complete remission have contributed to the improvement in overall survival for patients with this disease.<sup>21</sup> Using Surveillance, Epidemiology, and End Results Program (SEER) data after 1984, extensive analysis over the past three decades shows a progressive improvement in patient survival with HCL. Despite these truly remarkable results, at least 40 percent of patients will relapse.<sup>20</sup> Many of these patients will be successfully re-treated, but the failure of the disease-free survival curve to flatten attests to the fact that this disease has been controlled but not cured. Strategies to predict who will be prone to relapse will enable new treatments to be risk stratified. Furthermore, new agents are being developed to successfully treat patients who have developed resistant disease.<sup>20,25,26</sup>

## ● EPIDEMIOLOGY

HCL is a rare, chronic B-cell lymphoid malignancy accounting for approximately 2 percent of adult leukemias. The estimated annual incidence in the United States is approximately 3.0 persons per million person-years in the United States.<sup>27</sup> The mean age at diagnosis is 55 years, but there is a wide range in age of onset.<sup>20</sup> Patients may present in their 20s and 30s, and a report on 88 patients who were diagnosed at age 40 or younger showed that these patients do well long term.<sup>28</sup> Younger patients respond better to therapy but may relapse and require re-treatment to experience long-term survival benefits.<sup>9,20</sup> There is an unexplained male predominance with this disease, with a ratio of 4:1 males to females. There is also an unexplained racial difference, with more than 90 percent of patients being white.<sup>21</sup>

There is a bimodal presentation in age, raising the possibility of different etiologies in the younger and older patient groups.<sup>29</sup> The search for causative relationships in the older subset of patients has included extensive investigation of environmental and occupational exposures.<sup>30</sup> There is a suggestion of increased cases associated with farming and in jobs with extensive exposure to insecticides. Extensive investigation of the risk for secondary malignancies identified an increased overall risk (standardized incidence ratio [SIR] 1.24) in a large population-based study according to 1973 through 2000 SEER data.<sup>31</sup> In this study of more than 3000 patients with HCL, three separate malignancies were identified as of particular concern (SIR 6.61 Hodgkin lymphoma; SIR 5.03 non-Hodgkin lymphoma; and SIR 3.56 thyroid cancer). Whether these second malignancies are related to an immune deficit from the leukemia or a result of the therapy for the leukemia is unknown.

## ● ETIOLOGY AND PATHOGENESIS

HCL represents a clonal population of leukemic cells predominantly infiltrating the marrow, the spleen, and the liver. These cells are characterized as mature activated memory B cells based on their immunophenotypic profile.<sup>32</sup> The neoplastic cells in classic HCL (HCL-c) have hypermutated immunoglobulin genes in approximately 90 percent of the cases.<sup>33</sup> In those patients who have unmutated immunoglobulin genes, the disease appears to be more aggressive, as is the case in chronic lymphocytic leukemia. Leukemic cells use a variety of VH gene families. In those patients showing a *VH4-34* gene, the clinical course has also been noted to be less favorable.<sup>34,35</sup> Molecular parameters may have prognostic value. For example, patients with a mutation in *p53* have been less responsive to purine analogue therapy. Therefore, characterization of the molecular and genetic profile of the leukemic cells may elucidate the cell of origin and have prognostic value with respect to clinical outcome and responsiveness to standard therapy.

The identification of a mutation in *BRAF V600E* in almost 100 percent of patients with HCL-c has had a major impact on classification of the previously defined subsets of this disease.<sup>36</sup> The leukemic cells from patients with the variant of HCL (HCL-v) express *BRAF* wild type. This genetic difference confirms that these two entities are clearly unrelated, with a completely separate clinical course and therapeutic responsiveness. It is interesting that the small subset of patients with an immunophenotype consistent with HCL-c, yet using *VH4-34* rearrangement, appear to be *BRAF* wild type, further suggesting that there are multiple unique clonal patterns with distinct clinical courses.<sup>37</sup> Despite immunophenotypic features consistent with HCL-c, the lack of expression of *BRAF V600E* identifies a clinical course unlike the highly responsive form of the leukemia.

The presence of *BRAF V600E* mutation appears to activate the MEK-ERK (mitogen-activated protein kinase kinase–extracellular signal-regulated kinase) signaling pathway responsible for leukemic cell survival and proliferation. Downstream activation of pERK (phosphorylated ERK) correlates with presence of this mutational pathway.<sup>38</sup> Hematopoietic stem cells expressing this mutation have been identified in the marrow of murine models of the disease,<sup>39</sup> as well as in the marrow of patients with HCL.<sup>39</sup> *BRAF V600E* in marrow stem cells may explain the impairment in normal hematopoiesis that occurs in HCL-c. Furthermore, cytokines secreted by the leukemic cells may be responsible for the areas of hypocellularity observed in some patients with HCL-c.<sup>40,41</sup> Alternatively, impaired hematopoiesis may result from inadequate growth factor production.<sup>42</sup> Pancytopenia may also result from either extensive marrow infiltration with leukemia or fibrosis induced by the overproduction of transforming growth factor (TGF)  $\beta$  by leukemic cells.<sup>43</sup>

## ● CLINICAL FEATURES

The most common presenting symptoms of HCL are weakness and fatigue, which occur in 50 percent of patients.<sup>44</sup> Although many patients also have an enlarged spleen, the gradual onset of symptoms is described as fullness in the left abdomen, early satiety, and discomfort. Initially, Bouroncle reported that splenomegaly was found in 96 percent of patients.<sup>1</sup> However, more recently the percentage with a markedly enlarged spleen may be less at diagnosis because of earlier detection of the disease. Patients may present with a history of increased infections, and approximately 17 percent have an active infection at the time of diagnosis.<sup>1</sup> Bleeding manifestations are also noted in patients with severe thrombocytopenia. Patients may present with few symptoms but have an abnormal laboratory report suggesting a hematologic disorder may emerge during a routine health examination.



Infection has been the leading cause of death in patients with HCL and accounted for 55 percent of the fatalities in a large longitudinal review of 725 patients in an Italian series.<sup>45</sup> In general, the infections occur as a result of granulocytopenia, monocytopenia, and impaired function of immune effector cells. Approximately 30 percent of patients are found to have a documented source of infection, but an equal number of suspected infections cannot be documented microbiologically.<sup>46</sup> Fever in this patient population should prompt a search for infection. It has been estimated that 48 percent of infections are caused by pyogenic organisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Legionella pneumophila*.<sup>45,46</sup> Multiple other organisms have been identified as a source of infection, including *Aspergillus*, *Candida*, *Blastomyces*, *Histoplasma*, *Cryptococcus*, *Toxoplasmosis*, *Pneumocystis jiroveci*, and atypical mycobacteria.

Although the majority of infections occur before effective treatment has been initiated, the additional risk of infection as a complication of treatment exists both immediately following therapy and for many months thereafter.<sup>22</sup> The purine nucleoside analogues that are used as a backbone of induction therapy can produce profound and prolonged myelosuppression. Because of the extensive marrow involvement with leukemia, the myeloid reserve is severely compromised at the initiation of therapy. Following effective therapy, the granulocytes gradually recover, but the purine nucleoside analogues usually induce a prolonged period of reduction in lymphoid cells; thus, opportunistic infections resulting from compromised lymphocyte function may also emerge in the posttreatment period.<sup>47</sup> Once the patient has achieved a complete remission, the risks for infection become progressively less as the hematologic parameters improve. Full recovery of lymphocyte function following purine analogue therapy, however, may require several years.<sup>48,49</sup>

Unusual symptoms related to HCL may include bone pain and autoimmune complications.<sup>50,51</sup> Bone pain may be the result of lytic disease, which can involve the spine, the femur, and other skeletal sites. Lytic bone disease can occur at any time during the course of the disease. Both magnetic resonance imaging (MRI) and computed tomographic (CT) scans have been helpful in identifying these lesions even when plain films of involved areas are normal. Biopsies of bone lesions have confirmed the presence of hairy cells, and these manifestations may respond to effective treatment of the leukemia. Many of the patients with bone lesions respond to systemic treatment of the disease, but others require additional localized irradiation.

Diverse autoimmune findings in patients represent other unusual complications.<sup>51,52</sup> Patients may complain of migratory inflammatory episodes involving the joints and tenosynovial tissues. These painful inflammatory episodes are usually self-limited and resolve spontaneously but may be recurrent. Vasculitic skin lesions and erythema nodosum have been reported.<sup>53</sup> Autoimmune hemolytic anemia and thrombocytopenia have also been observed.<sup>54–56</sup> The autoimmune phenomena are not related to tumor burden and may be present at initial diagnosis or occur any time throughout the course of the disease. Finally, patients have also presented with paraneoplastic neurological syndromes.<sup>57</sup>

## ● LABORATORY FEATURES

Patients with HCL reviewed in a large Italian series had pancytopenia (77 percent), reflecting impaired hematopoiesis due to marrow infiltration and splenic sequestration.<sup>45</sup> Approximately 28.4 percent of patients had a hemoglobin value less than 8.5 g/dL, with 14.9 percent requiring a blood transfusion; 39 percent of patients had absolute neutropenia (neutrophils <500/ $\mu$ L), and 72.6 percent had a platelet

count less than 100,000. Jansen earlier developed prognostic criteria for HCL relating the degree of anemia and splenomegaly to survival.<sup>58</sup> The Jansen staging system was developed before the age of effective therapy. Nevertheless, a later study also showed that the degree of anemia in younger patients correlated with overall survival following therapy with pentostatin.<sup>9</sup>

Most patients have monocytopenia. Many patients have morphologic evidence of “hairy cells” on blood films; these cells are characterized by pale blue or gray cytoplasm with a serrated/ruffled border (Fig. 4-1). The nucleus is oval and often reniform in shape, with spongy chromatin and indistinct nucleoli. In the past, cytochemical staining with tartrate-resistant acid phosphatase (TRAP) was routinely performed because hairy cells are positive for this enzyme. However, this stain is technically difficult and was therefore replaced by immunohistochemical stains (IHC) for this enzyme. Moreover, a more definitive diagnosis can be made by flow cytometry to identify the characteristic immunophenotypic profile. HCL cells are strongly positive for CD20 and are positive for CD11c+, CD25+, CD103+, and CD123+. The leukemic cells are usually negative for CD5–, CD10–, CD27–, and CD43–. Consequently, it is essential to secure a comprehensive immunophenotypic profile of the leukemic cells, as even small monoclonal populations can be identified by multichannel flow cytometry in the blood (Fig. 4-2).

## MARROW BIOPSY

In establishing the diagnosis, a marrow aspirate and biopsy should be obtained to evaluate the degree of marrow cellularity and the percentage of leukemic cell infiltration (Fig. 4-1).<sup>59</sup> In addition, marrow fibrosis is characteristic of this disease, and a biopsy is particularly important as the aspirate may be inadequate in the presence of fibrosis. The marrow cellularity can be quite variable. Some patients with this diagnosis have a severely hypocellular marrow, and this pattern could be misread as hypocellular or aplastic anemia.<sup>59</sup> More often, there is either infiltrative disease or diffuse marrow replacement with the characteristic mononuclear cells with nonoverlapping cellular borders. The appearance of these cells has resembled a “fried egg” (Fig. 4-1). IHC can be used to definitively identify the leukemic cells within the biopsy. Immunohistochemical staining of the marrow with anti-CD20 is most useful, followed by staining with annexin and DBA.44 monoclonal antibody to further narrow the differential diagnosis (Fig. 4-3). The demonstration that the mutation *BRAF V600E* is found in the overwhelming majority of cells from patients with HCL-c has provided yet another confirmatory stain for this disease.<sup>60</sup>

Additional baseline laboratory tests to obtain before treatment include an assessment of renal function as both of the commonly used purine nucleoside analogues are excreted by a renal route. It is important to screen for evidence of previous hepatitis B as serious complications, including acute liver injury, have resulted from the use of immunosuppressive agents (e.g., rituximab).<sup>61</sup>

## LABORATORY VALUES USEFUL IN PATIENT MONITORING

Serial complete blood cell count monitoring with attention to the absolute neutrophil count, the platelet count, and the hemoglobin is the most useful approach to follow the progress of patients with HCL. As the expression of soluble interleukin 2 (IL-2) receptor correlates with tumor burden, a baseline determination of the soluble IL-2 receptor may be important for following the course of the disease and its response to treatment.<sup>62</sup> Soluble CD22 also can be followed in a similar manner as a correlate of leukemic cell burden.<sup>63,64</sup>

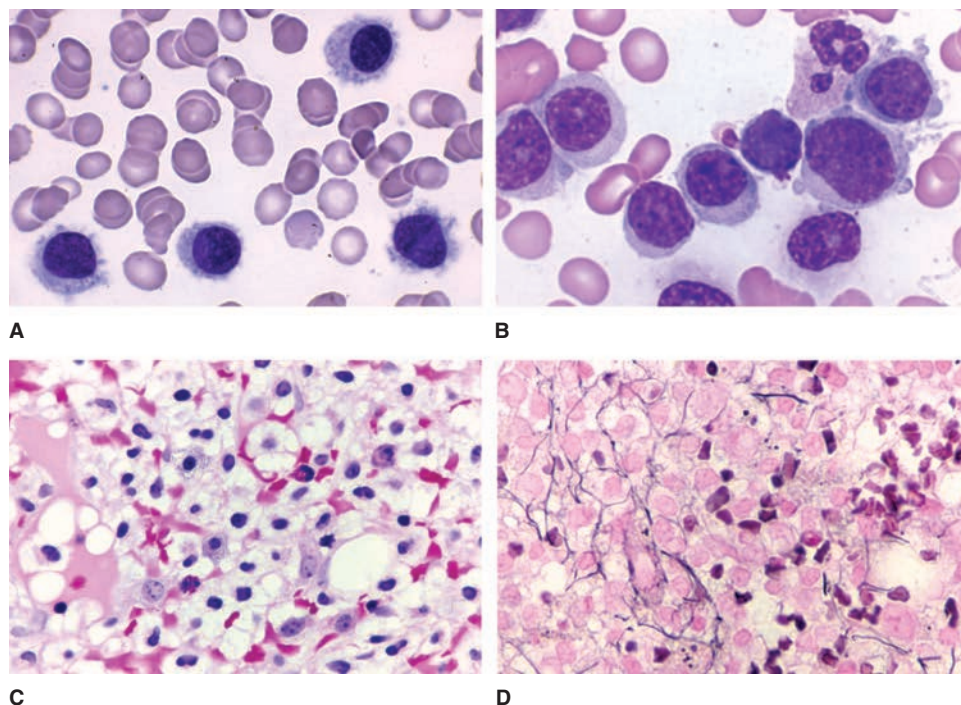


**Figure 4-1.** Hairy cell leukemia (HCL). **A.** Blood film showing four hairy cells with characteristic abundant pale blue or gray cytoplasm with ruffled margin (“hairs”). By electron microscopy, the hairs are narrow projections approximately  $0.50\ \mu\text{M}$  in length. **B.** Marrow aspirate showing infiltrate of hairy cells. **C.** Marrow biopsy showing the so-called fried egg appearance generated by the spacing of the lymphocytic infiltrate and the abundant hairy cell cytoplasm, leaving the nucleus to simulate the yolk of a fried egg. **D.** Silver stain demonstrating an increase in argentophilic, type III collagen (reticulin), commonly present in the marrow in this disease. This reactive fibrosis accounts for the frequency of acellular marrow aspirates and the importance of a concomitant marrow biopsy for diagnosis. (Reproduced with permission from Lichtman’s Atlas of Hematology, <http://accessmedicine.com>.)

## DIFFERENTIAL DIAGNOSIS

Several B-cell clinical entities can be considered when establishing a diagnosis of HCL-c. The World Health Organization (WHO) defined an HCL-v that is completely separate from the classic form of this disease.<sup>20,41,60,65</sup> The frequency of HCL-v is approximately 10 percent of HCL-c.<sup>65</sup> This rare, chronic, B-cell lymphoproliferative disorder is characterized by leukocytosis, lack of monocytopenia, and neoplastic B cells with nucleoli and convoluted nuclei. The cytoplasm may have a shaggy edge. The immunophenotype is characterized as CD25– and CD123– negative, annexin-1 negative, and negative for TRAP, both by cytochemical and by immunohistochemical methods. The *BRAF V600E* mutation is not present in this entity.<sup>36</sup> The clinical course of the disease is initially indolent, but eventually progresses with spleen and liver involvement. Some patients respond to splenectomy with temporary stabilization of the disease. Patients do not achieve durable responses with purine analogues as monotherapy but may respond to combined therapies with a purine analogue and a monoclonal antibody.<sup>66,67</sup>

Another entity that must be distinguished from HCL is splenic marginal zone lymphoma/splenic marginal zone lymphoma with villous lymphocytes.<sup>65</sup> This entity is a chronic B-cell neoplasm that involves the spleen, splenic hilar nodes, marrow, and the blood. Patients may present with splenomegaly, anemia, and thrombocytopenia. The malignant cells in the blood are characterized by cytoplasmic villi/projections that are typically polar in distribution. The immunophenotypic profile is distinctly different from HCL-c. While the cells are positive for CD20, they are negative for CD25 and annexin 1 and usually negative for CD103.

Splenic, diffuse, red pulp, small B-cell lymphoma is an uncommon lymphoma that infiltrates the splenic red pulp in a diffuse pattern.<sup>65</sup> It

can also involve the marrow and the blood. The malignant cells resemble those seen in splenic marginal lymphoma with villous lymphocytes. Patients usually have very large spleens, leukopenia, and thrombocytopenia. The immunophenotypic profile shows strong expression of CD20 and negative staining for CD25, CD11c, CD123, and annexin. This indolent lymphoma has been reported to respond to splenectomy.

There are patients with classic-appearing HCL who have molecular features suggesting that there may be more than one molecular “variant” of this disease. For example, patients with a classic immunophenotype who are *BRAF V600E* mutation-negative and use the immunoglobulin *VH4-34* have a worse prognosis than those with HCL-c, despite having identical immunophenotypic markers.<sup>20,37</sup> Patients with unmutated immunoglobulin gene rearrangement and those harboring a p53 mutation also have a worse prognosis, indicating that these molecular features potentially define yet another form of this disease.<sup>33</sup> It is hoped further study of the molecular prognostic features will identify information that will enable improvement in selection of appropriate therapy.<sup>20</sup> Establishing an accurate diagnosis of HCL or one of the clinical entities that mimic this disease by including a complete set of immunophenotypic and molecular markers is essential in selecting the best therapeutic option for each patient (Table 4-1).

## THERAPY

### CRITERIA FOR INITIATION OF TREATMENT

Patients with HCL should be treated for symptoms related to the disease or for deterioration in blood cell counts. Patients may have symptoms associated with a markedly enlarged spleen. Excessive fatigue related

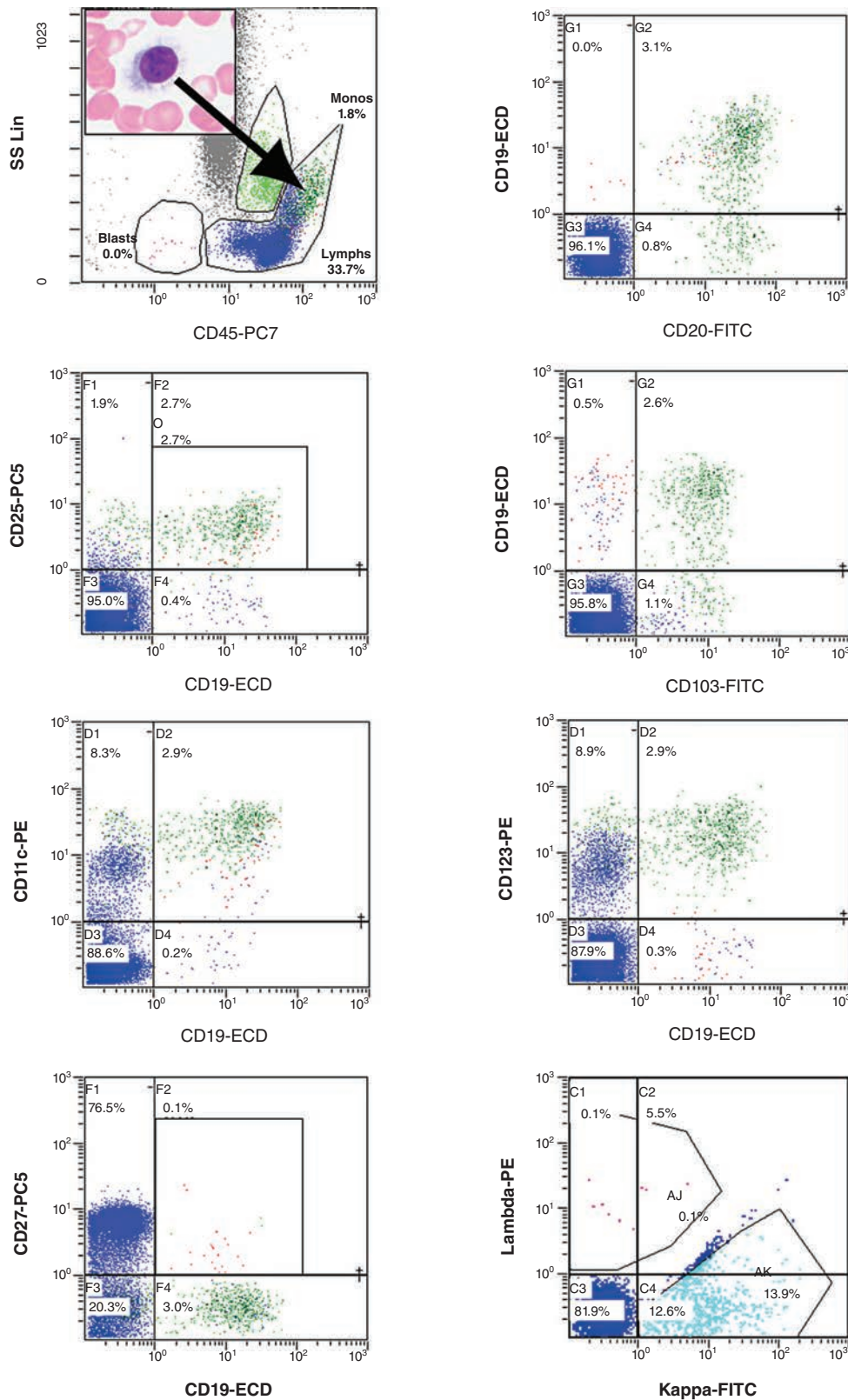


**Figure 4-2.** Hairy cell leukemia (HCL) immunophenotypic profiling should be performed using multiparametric flow cytometry analysis. Both blood and hemodilute marrow aspirates should be analyzed regardless of the number of leukemic cells seen on morphologic review of slides. Because of a characteristic immunophenotype, definitive diagnosis of HCL can be rendered based on flow cytometric data even with a very low number of leukemic cells. As a result of their complex surface projections, hairy cells demonstrate moderate-to-high side scatter (**SS**) characteristics, resulting in their shift on flow plots into the region typically occupied by monocytes. Hairy cells are positive for CD11c+, CD19+, CD20+, CD25+, CD103+, and CD123+ and show  $\kappa$  light-chain restriction in this example. Hairy cells are negative for CD27-. Rare cases of HCL may show positive staining for CD5 or CD10 antigens. It is important to note that, while atypical, the expression of these antigens does not preclude a definitive diagnosis of HCL in cases with otherwise typical HCL immunophenotype. ECD, electron coupled dye; FITC, fluorescein isothiocyanate.



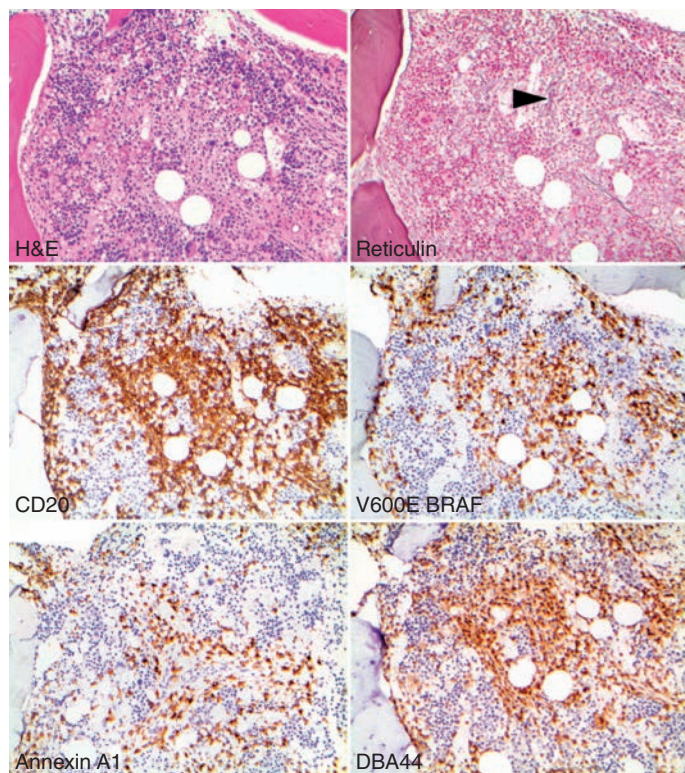


**Figure 4-3.** Marrow core biopsy is important in diagnosis of hairy cell leukemia (HCL) because marrow aspirates are frequently dry taps in HCL. This figure shows a marrow trephine biopsy involved by HCL. Hematoxylin-and-eosin (H&E)-stained sections show a characteristic interstitial pattern of marrow infiltration by HCL. Leukemic cells are medium size with ample clear cytoplasm and centrally placed nuclei without nucleoli with “fried egg” morphology. Leukemic cells do not form discrete aggregates but are admixed with hematopoietic elements, which often render their distinction from background erythroid precursors difficult. *Reticulin* stain shows reticulin fibrosis that is characteristically present in marrow involved by HCL and renders marrow difficult to aspirate, resulting in frequent dry tap aspirates. *CD20* immunohistochemical stain highlights the extent of leukemic infiltrate. *V600E BRAF* immunohistochemical stain highlights leukemic cells that typically are positive for *V600E BRAF* mutation. *Annexin A1* immunohistochemical stain is positive in leukemic cells and is negative in background residual erythroid cells. *DBA44* immunohistochemical stain, while not absolutely specific, is always positive in HCL.

either to the underlying disease or to the degree of anemia also warrants treatment. If the absolute neutrophil count is documented to be less than  $1000/\mu\text{L}$  or if the platelet count is confirmed to be less than  $100,000/\mu\text{L}$ , then consideration for treatment should be given rather than waiting until the patient's blood counts have deteriorated to very low levels. Many patients will have achieved these low hematologic parameters by the time of diagnosis, therefore meriting prompt therapy. Approximately 10 percent of patients with HCL-c may not meet these criteria and can be followed for an extended period of time without therapy, albeit with close followup.<sup>64,66,68,69</sup>

If patients have had recurrent infections requiring antibiotics, it may be prudent to delay treatment for the HCL until after the infection has been controlled. After the infection is controlled, subsequent treatment with a purine analogue can be administered to secure a consolidated remission of the leukemia. These challenges highlight the importance of starting effective antileukemic therapy before the absolute neutrophil count deteriorates to a dangerous level.

## STANDARD APPROACH

Patients may be treated with either cladribine or pentostatin (Table 4-2).<sup>64,70,71</sup> Cladribine has been approved for initial therapy, and pentostatin has been approved for second-line therapy. Cladribine has been administered by several routes on differing schedules. This agent is usually administered over 5 to 7 days, and the patient's blood cell counts need to be carefully monitored after this initial course. Initial studies administered cladribine as a continuous intravenous infusion for 7 days as a single course.<sup>10</sup> Subsequently, other investigators administered this agent as a daily intravenous infusion over 2 hours each day for 5 days.<sup>71</sup> Subcutaneous administration of cladribine has also been successful and may be more convenient.<sup>72,73</sup> Approximately 4 to 6 months after blood cell count recovery following cladribine, a marrow biopsy should be obtained to determine the quality of the response. The overall complete remission rate with this agent is reported to vary from 75 to 91 percent.<sup>70,74,75</sup>

Alternatively, if pentostatin is to be used for induction therapy, it is administered once every 2 weeks as a short intravenous injection followed by administration of approximately a liter of fluid.<sup>9,64</sup> Weekly blood cell counts are monitored, and the second and subsequent doses are administered if the absolute granulocyte count has not decreased to dangerously low levels. Titrating these doses to be given every 2 to 3 weeks may lessen the degree of myelosuppression related to the agent.<sup>64</sup> After several reduced or delayed doses, the dose and schedule are returned to the standard dose of  $4 \text{ mg/m}^2$  given intravenously every 2 weeks in an effort to achieve complete remission. The complete remission rate to pentostatin with this approach approximated 75 percent in a multi-institutional study.<sup>9</sup> Patients may require 6 months or more of therapy with this agent. When the blood cell counts and the spleen have returned to normal, then a marrow biopsy should be performed to see if complete remission has been achieved by morphologic evaluation. This biopsy will serve as a baseline for evaluation of MRD. If there are no visible areas of HCL by morphologic criteria, then two additional doses are administered as consolidation.

## MINIMAL RESIDUAL DISEASE

Although a complete remission is based on recovery of blood cell counts, there must be no morphologic evidence of leukemic cells in either the blood or the marrow.<sup>20</sup> MRD is defined as evidence of leukemic cells on the marrow biopsy that can be detected using IHC when there is no residual morphologic evidence of disease. IHC directed at markers on the leukemic cells may identify residual disease that is either diffusely infiltrating the marrow or localized. Antibodies directed at CD20, *BRAF V600E* (e.g., VE1), or DBA.44 will detect disease that is not identified morphologically.<sup>20</sup> In addition, detailed flow cytometric immunophenotypic analysis of either the blood or the marrow aspirate may be capable of identifying residual leukemia cells (e.g., positive for CD20+, CD11c+, CD103+, CD25+, CD123+ and negative for CD27-). Flow cytometry of the marrow may be negatively affected by the difficulty in securing an aspirate that is not contaminated by blood. Consequently, identification of the extent of MRD using IHC on a marrow biopsy may be less adversely affected by sampling error. Eradication of MRD may be achieved by adding additional therapy (e.g., administration of rituximab), but the necessity for this additional therapy must be considered.<sup>76</sup>

## FOLLOWUP CARE

The advantages of achieving a complete remission have been stressed.<sup>77</sup> However, the therapy for HCL is immunosuppressive. Extensive treatment to eradicate MRD may involve continued therapy with its

**TABLE 4-1.** Differential Diagnosis for Hairy Cell Leukemia

| Characteristics                             | HCL                                                                                        | HCL-v                                                                                                           | SMZL                                                                                                    | SDRPSBL                                                                                    |
|---------------------------------------------|--------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|
| Number of circulating malignant cells       | Low                                                                                        | Moderate                                                                                                        | Variable                                                                                                | Low                                                                                        |
| Monocytopenia                               | Present                                                                                    | Absent                                                                                                          | Absent                                                                                                  | Absent                                                                                     |
| Chromatin                                   | Open                                                                                       | Condensed                                                                                                       | Condensed                                                                                               | Condensed                                                                                  |
| Nucleolus                                   | Absent                                                                                     | Prominent                                                                                                       | Absent                                                                                                  | Variable                                                                                   |
| Cytoplasm                                   | Abundant with prominent circumferential hairy projections                                  | Moderate to abundant with variably prominent circumferential hairy projections                                  | Moderate to scant with variably prominent polar hairy projections                                       | Moderate with variably prominent villous projections                                       |
| Spleen involvement                          | Red pulp                                                                                   | Red pulp                                                                                                        | White pulp                                                                                              | Red pulp                                                                                   |
| Marrow involvement                          | Interstitial, diffuse pattern (fried egg morphology)<br>Marrow reticulin fibrosis          | Sinusoidal may be interstitial                                                                                  | Nodular may be intrasinusoidal                                                                          | Intrasinusoidal, may be interstitial or nodular                                            |
| Marrow reticulin fibrosis                   | Frequent and marked                                                                        | Absent                                                                                                          | Absent                                                                                                  | Absent                                                                                     |
| Immunophenotype by flow cytometric analysis | CD11c+, CD19+, CD20+ bright, CD22+, CD25+, CD103+, CD123+, FMC7+, kappa or lambda (strong) | CD11c+, CD19+, CD20+, CD22+, CD27+, CD79b+, CD103+, FMC7+, kappa or lambda strong<br>Negative for CD25-, CD123- | CD11c+, CD19+, CD20+, CD22+, CD27+, CD79b+, FMC7+, kappa or lambda strong<br>Negative for CD25-, CD123- | CD11c+/-, CD103+/-, CD19+, CD20+, kappa or lambda+<br>Negative for CD25-, CD123-           |
| Immunophenotype by immunohistochemistry     | DBA.44+<br>Annexin A1+<br>Immuno-TRAP+<br>Cyclin D1+<br>Faint t-Bet+<br>V600E BRAF+        | DBA.44+<br>Annexin A1-<br>Immuno-TRAP-<br>Cyclin D <sub>1</sub> -<br>t-Bet-<br>V600E BRAF-                      | DBA.44+/-<br>Annexin A1-<br>Immuno-TRAP-<br>Cyclin D <sub>1</sub> -<br>t-Bet-<br>V600E BRAF-            | DBA.44+<br>Annexin A1-<br>Immuno-TRAP-<br>Cyclin D <sub>1</sub> -<br>t-Bet-<br>V600E BRAF- |
| Recurrent mutation                          | V600E BRAF                                                                                 | None                                                                                                            | None                                                                                                    | None                                                                                       |
| Somatic hypermutation of immunoglobulin     | >85% of cases                                                                              | Mostly                                                                                                          | >50% of cases                                                                                           | Variable                                                                                   |

HCL, hairy cell leukemia; HCL-v, variant of hairy cell leukemia; SDRPSBL, splenic diffuse red pulp small B-cell lymphoma; SMZL, splenic marginal zone lymphoma; t-Bet, T-box transcription factor; TRAP, tartrate-resistant acid phosphatase.

attendant consequences of increased risk for infection or possibly a secondary malignancy. Consequently, clinical judgment must be exercised to achieve the optimal outcome for patients with this disease. An accurate assessment of response to therapy may best be made several months following completion of initial induction therapy.<sup>76</sup> In those who have achieved a complete response, careful followup is indicated. In those who had less than a complete remission, a determination will need to be made regarding salvage therapy versus close observation based on blood cell count recovery.

## THERAPY AT RELAPSE

The duration of response following initial therapy for HCL is variable. The criteria for re-treatment can be based on recurrence of clinical symptoms and on the status of the blood cell count.<sup>69</sup> A decision to restart therapy before progressive severe pancytopenia has returned is important. Considering both the risks and the benefits of additional therapy requires clinical judgment.

There are several therapeutic options for treating patients with either resistant disease or disease that had an early relapse following initial response. In general, if the patient achieved an initial response and

subsequently relapses within 1 to 2 years, an alternate agent might be selected for re-treatment.<sup>64</sup> Alternatively, reinduction therapy may also include a combination of a purine analogue and a monoclonal antibody. In contrast, re-treatment with the initial therapy can be considered if the first remission was durable. Most patients will initially be treated with a purine nucleoside analogue. However, long-term durable remissions are being observed in patients being treated with cladribine followed by rituximab. The necessity for combined chemoimmunotherapy as initial therapy requires further investigation<sup>78</sup>

In patients with HCL-c who demonstrate resistant disease, the identification of the *BRAF* V600E target has provided a therapeutic strategy involving an inhibitor (e.g., vemurafenib) as investigational therapy. Although patients in several studies have been reported to respond, this agent is not yet approved by the FDA for this indication.<sup>25,26</sup> Furthermore, immunotoxin conjugates have also been reported to produce remissions in patients with resistant disease.<sup>79</sup> The purine nucleoside analogues (cladribine and pentostatin) have also been effective in producing long-term salvage remissions in patients with HCL in relapse. In patients who have either resistant or relapsed disease, a combination of a purine analogue and a monoclonal antibody have also been used.<sup>64,76</sup>



**TABLE 4-2.** Management of Hairy Cell Leukemia

Determine accurate diagnosis

- Marrow biopsy with immunohistochemical analysis
- Blood immunophenotypic characterization

Decision on initiation of therapy

- Approximately 10 percent can be carefully followed on “watch-and-wait” approach but majority of patients require treatment
- Determinants or symptoms prompting treatment: symptomatic splenomegaly or laboratory studies showing absolute neutrophil count <1000/μL; hemoglobin <10 g/dL; or platelet count <100,000/μL

Important assessments before therapy for leukemia

- Presence of or suspicion for infection
- Adequate renal function
- Previous exposure to hepatitis

Decision on frontline therapy

- Cladribine 0.1 mg/kg/day for 7 days continuous intravenous infusion<sup>12,74,81</sup>
- Cladribine 0.12 mg/kg/day for 5 days as 2-hour intravenous infusion vs. weekly infusion for 6 weeks<sup>14</sup>
- Pentostatin 4 mg/m<sup>2</sup> intravenous dose every 2 weeks until maximal response or failure<sup>9,77,82</sup>

Assessment of response

- Following induction therapy, a marrow biopsy to document quality of response and quantitate minimal residual disease (MRD)
- Methods for quantification of MRD with immunohistochemical stains and the optimal timing for MRD assessment are under investigation
- In general, response assessment after cladribine is recommended after 3 to 5 months. In contrast, response assessment following pentostatin is made at time of best clinical response

Clinical investigations for resistant hairy cell leukemia

- Alternate purine analogues alone or combined chemoimmunotherapy (e.g., bendamustine and rituximab)<sup>83</sup>
- Immunotoxin conjugates (e.g., moxetumomab pasudotox [HA22]<sup>79</sup>)
- *BRAF V600E* inhibitors (e.g., vemurafenib)<sup>26,84</sup>

Overall management strategies can be found in Refs. 64, 70, 76, and 85.

## COURSE AND PROGNOSIS

The outlook for patients with HCL has markedly improved since its original 1958 description.<sup>71</sup> Patients can now anticipate a near-normal life expectancy, with the caveat that the disease will likely require close observation and re-treatment for those who relapse. Survival at the end of 1 year is estimated to be approximately 88 percent, with 5-year survival at 77 percent in one longitudinal population-based report.<sup>22</sup> Other long-term followup studies in the era of purine nucleoside therapy have identified similar results, with 5-year survival being 90 percent and estimated 10-year survival at 81 percent.<sup>77</sup>

Whereas overall survival has improved, there is an increased risk of serious infection during the first year following diagnosis. The overall relative risk of a serious infection compared to a normal population is 2.59. This adjusted relative risk is 8.04 during the first year from diagnosis and treatment.<sup>22</sup> The risk of serious infection is highest during the

first year and then declines toward normal in subsequent years. This indicates that patients should be followed closely during the initial years following treatment. Full recovery of lymphocyte numbers and function after therapy may require several years. Consequently, physicians should follow their patients closely and document the recovery of these immune effector cells. Patients should receive vaccinations utilizing dead or attenuated viral vaccines and avoid “live” viral vaccines while in remission. Prompt attention to early signs of infection is important for health maintenance.

The results of clinical investigations have markedly improved the outcome for this patient population. Clinical relapse will likely occur because the current therapy controls the disease but does not cure it.<sup>80</sup> Despite the enormous progress made in managing these patients, continued clinical investigation is warranted in an effort to achieve the best outcome, with durable complete remissions and minimal risk of infection. Consensus guidelines for the diagnosis and management of patients with HCL-c were recently developed.<sup>69</sup> It is hoped this will serve as a foundation for continued improvement in management for these patients.

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## CHAPTER 5

# LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

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### SUMMARY

Indolent clonal proliferations of large granular lymphocytes (LGLs) can arise from either T cells or natural killer (NK) cells. These diseases show overlapping clinical, morphologic, immunophenotypic, and genetic features. T-cell large granular lymphocytic leukemia (T-LGL) and the related provisional 2008 World Health Organization entity chronic lymphoproliferative disorders of NK cells (CLPD-NK) are similarly defined as persistent (>6 months) and with clonal expansions in blood LGLs, often without a clearly identifiable cause. Patients with these diseases are typically older, present with single-lineage or multilineage cytopenias, and often have clinical and laboratory features of autoimmunity or immune dysfunction. Autoimmune neutropenia, thrombocytopenia, hemolytic anemia, and occasionally pure red cell aplasia may occur. Patients with T-LGL frequently have elevated rheumatoid factor and clinical hallmarks of rheumatoid arthritis. The diagnosis of LGL requires a high degree of suspicion and careful examination of the blood film because a significant fraction of patients do not have an absolute lymphocytosis, although the proportion of LGLs is usually increased. Most patients with T-LGL and fewer with CLPD-NK have chronic neutropenia, and approximately half of patients with T-LGL have neutrophil counts less than  $0.5 \times 10^9/L$ . Anemia is observed in approximately half of patients with T-LGL. Morbidity and mortality usually result from recurrent infections secondary to chronic neutropenia or transfusion-related iron overload and less frequently from disease acceleration and transformation into a more aggressive T/NK leukemia or lymphoma. The treatment approach generally consists of immune-modulatory or immune-suppressive drugs, such as weekly oral methotrexate, cyclophosphamide, cyclosporine, prednisone, and alemtuzumab. Prospective studies are examining the role of these agents in LGL.

**Acronyms and Abbreviations:** AICD, activation-induced cell death; ANKL, aggressive NK cell leukemia; BCR-ABL, breakpoint cluster region-Abelson; CD, cluster of differentiation; CDR3, complementarity determining region 3; CLPD-NK, chronic lymphoproliferative disorders of NK cells; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; FLT3, FMS-like receptor tyrosine kinase-3; FS, Felty syndrome; HLA, human leukocyte antigen; HSTCL, hepatosplenic T-cell lymphoma; HTLV, human T-cell leukemia virus; IL, interleukin; ITD, internal tandem duplication; JAK, Janus kinase; KIR, killer immunoglobulin-like receptor; LGL, large granular lymphocyte; LGLL, large granular lymphocytic leukemia; NK, natural killer cell; NK-LGL, natural killer cell—large granular lymphocyte; PDGF, platelet-derived growth factor; PI3K, phosphatidylinositol 3'-kinase; RF, rheumatoid factor; STAT, signal transducer and activator of transcription; TCR, T-cell receptor; T-LGL, T-cell large granular lymphocytic leukemia; WHO, World Health Organization.

### DEFINITION AND HISTORY

Large granular lymphocytic leukemia (LGL) was initially described in the 1970s,<sup>1,2</sup> and in 1985 further characterized,<sup>3</sup> as a clonal disorder of cytotoxic cluster of differentiation (CD)8+ T cells involving blood, marrow, liver, and spleen and clinically manifesting as an indolent proliferation of large granular lymphocytes (LGLs). Normally, LGLs comprise 10 to 15 percent of blood mononuclear cells and may be either surface CD3+ (T-cell) or surface CD3- (natural killer [NK] cell). The absolute number of LGLs in the blood of normal subjects is  $0.2$  to  $0.4 \times 10^9/L$ .

According to the 2008 WHO [World Health Organization] *Classification of Tumours of the Haematopoietic and Lymphoid Tissues*, T-cell large granular lymphocytic leukemia (T-LGL) is defined as a persistent (>6 months) and usually clonal expansion of surface CD3 (sCD3+) LGLs without a clearly identifiable cause.<sup>4</sup> The corresponding NK cell type of LGL (sCD3-, CD16+), referred to as chronic lymphoproliferative disorders of NK cells (CLPD-NK), was included as a provisional diagnosis in the 2008 WHO classification and is similarly defined.<sup>5</sup> LGL represents 2 to 3 percent of mature lymphocytic leukemias.<sup>4</sup> CLPD-NK should be distinguished from the acute and often fulminant aggressive NK cell leukemia (ANKL),<sup>6</sup> which is associated with Epstein-Barr virus (EBV) infection of the neoplastic NK cells. In contrast to ANKL, both T-LGL and CLPD-NK are clinically indolent and have a low risk of transformation into an aggressive malignancy. The main impact of LGL on patients' lives, and the most common indication for therapy, derives from the occurrence and severity of single-lineage or multilineage cytopenias and by the resultant infections and transfusion requirement.

### ETIOLOGY AND PATHOGENESIS

The etiologies of T-LGL and CLPD-NK are not definitively established. It has been postulated, based on the analysis of T-cell receptor (TCR) complementarity determining region 3 (CDR3) patterns and V $\beta$  family usage, that chronic antigenic stimulation results in the proliferation or increased survival of LGLs. Moreover, leukemic T-LGL shows characteristics of antigen-activated cytotoxic T lymphocytes (CTLs), suggesting that an initial step in T-LGL could be an antigen-driven clonal expansion.<sup>7-9</sup> Clonal drift in the T-cell repertoire with a change in the dominant clone occurs in approximately one-third of cases during the course of the disease,<sup>10</sup> implying that a dynamic process of clonal expansion exists that may affect more than one T-cell family in the same patient. Early studies suggested a possible association with human T-cell leukemia viruses (HTLVs); however, most patients are not infected with this agent.<sup>11</sup> Some evidence implicates cytomegalovirus (CMV) as the inciting antigen in the rare CD4+ subset of LGL, but its role in patients with the more typical CD8+ subtype of T-LGL is not clear.<sup>12</sup>

In the absence of an exogenous antigenic drive, chronic immune dysregulation and aberrant cytokine production may lead to enhanced LGL survival and expansion and therefore contribute to the pathogenesis of LGL. Patients with T-LGL frequently have humoral immune abnormalities, including positive tests for rheumatoid factor (RF)—with or without clinical arthrosynovitis—antinuclear antibodies, antineutrophil cytoplasmic antibodies, polyclonal hypergammaglobulinemia, hypogammaglobulinemia, and circulating immune complexes (Table 5-1).<sup>13</sup> The high incidence of autoimmunity in patients with LGL and the fact that the autoimmune manifestations often precede the occurrence of LGL expansions suggest that sustained immune activation may contribute to the pathogenesis of LGL. However, because the detection of asymptomatic expansions of LGLs in the blood requires examination of a blood film or flow cytometry, this temporal association should be interpreted with caution.



**TABLE 5-1.** Clinical Features of T-Cell Large Granular Lymphocytic Leukemia

|                                      | Pandolfi <sup>68</sup><br>(1990) | Loughran <sup>69</sup><br>(1993) | Dhodapkar <sup>70</sup><br>(1994) | Semenzato <sup>71</sup><br>(1997) | Neben <sup>72</sup><br>(2003) | Bareau <sup>48</sup><br>(2010) |
|--------------------------------------|----------------------------------|----------------------------------|-----------------------------------|-----------------------------------|-------------------------------|--------------------------------|
| Number of patients                   | 151                              | 129                              | 68                                | 162                               | 44                            | 201                            |
| Median age                           | 55                               | 57                               | 61                                | 59                                | 63                            | 59                             |
| M/F                                  | 1.3                              | 0.8                              | 1                                 | 0.8                               | 1.0                           | 0.8                            |
| Symptomatic                          | 72%                              | –                                | 69%                               | –                                 | 73%                           | 82%                            |
| Splenomegaly                         | 50%                              | 50%                              | 19%                               | 50%                               | 35%                           | 24%                            |
| Hepatomegaly                         | 34%                              | 23%                              | 1%                                | 32%                               | –                             | 10%                            |
| Adenopathy                           | 13%                              | 1%                               | 3%                                | 13%                               | 5%                            | 6%                             |
| B symptoms                           | –                                | –                                | 12%                               | –                                 | –                             | 7%                             |
| Infections                           | 38%                              | 39%                              | 15%                               | 56%                               | –                             | 23%                            |
| Rheumatoid arthritis                 | 12%                              | 28%                              | 26%                               | 36%                               | 20%                           | 17%                            |
| Rheumatoid factor                    | –                                | 57%                              | 61%                               | 43%                               | 48%                           | 41%                            |
| Antinuclear antibodies               | –                                | 38%                              | 44%                               | 38%                               | 48%                           | 48%                            |
| Autoimmune cytopenias                | –                                | –                                | 7%                                | 9%                                | 5%                            | 7%                             |
| Lymphocytosis                        |                                  |                                  | 29%                               |                                   |                               |                                |
| LGL >4 × 10 <sup>9</sup> /L          | 52%                              | 52%                              | –                                 | –                                 | –                             | 14%                            |
| LGL 1–4 × 10 <sup>9</sup> /L         | 38%                              | 40%                              | –                                 | –                                 | –                             | 50%                            |
| LGL <1 × 10 <sup>9</sup> /L          | 10%                              | 8%                               | –                                 | 7%                                | –                             | 36%                            |
| Neutropenia                          |                                  |                                  |                                   |                                   |                               |                                |
| Moderate (<1.5 × 10 <sup>9</sup> /L) | 64%                              | 84%                              | 74%                               | –                                 | 52%                           | 61%                            |
| Severe (0.5 × 10 <sup>9</sup> /L)    | 7%                               | 48%                              | 40%                               | 37%                               | 41%                           | 26%                            |
| Anemia                               |                                  |                                  |                                   |                                   |                               |                                |
| Any severity                         | 25%                              | 49%                              | 51%                               | 26%                               | 89%                           | 24%                            |
| Severe (Hb <8 g/dL)                  | 37%                              | –                                | 19%                               | –                                 | 36%                           | 7%                             |
| Thrombocytopenia                     | 9%                               | 19%                              | 20%                               | 29%                               | 36%                           | 19%                            |
| LGL marrow infiltration              | 67%                              | 88%                              | –                                 | 76%                               | 83%                           | 72%                            |
| Hypergammaglobulinemia               | –                                | 45%                              | 5%                                | 43%                               | –                             | 35%                            |
| Monoclonal gammopathy                | –                                | 45%                              | 8%                                | –                                 | –                             | 10%                            |
| Need for treatment                   | 30%                              | 73%                              | 69%                               | 33%                               | 80%                           | 44%                            |
| LGLL-related death                   | 14%                              | 36%                              | 8%                                | 27%                               | –                             | 7%                             |

Hg, hemoglobin; LGL, large granular lymphocyte; LGLL, large granular lymphocytic leukemia; M/F, male-to-female ratio.

Modified with permission from Bareau B, Rey J, Hamidou M, et al: Analysis of a French cohort of patients with large granular lymphocyte leukemia: A report on 229 cases. *Haematologica* 2010 Sep;95(9):1534-1541.

Normal CTL homeostasis is maintained, in part, through activation-induced cell death (AICD). Leukemic T-LGLs constitutively express high levels of Fas (CD95) and Fas ligand (CD178), yet are resistant to Fas-mediated death.<sup>14</sup> Some disease manifestations, such as neutropenia, are associated, at least in part, with circulating CD178 in these patients.<sup>15</sup> High levels of pro-inflammatory or prosurvival cytokines associated with sustained immune activation could account for at least part of the mechanism driving LGLs.<sup>16–19</sup> Likewise, constitutive activation of survival signaling pathways could represent a central pathogenetic mechanism in LGLL. Evidence for the importance of signal transducer and activator of transcription (STAT) 3/Mcl-1, phosphatidylinositol 3'-kinase (PI3K)/AKT, and sphingolipid signaling leading to apoptotic resistance has been demonstrated for all.<sup>20–22</sup> Mutations in STAT3 were

identified in approximately 40 percent of T-LGLL and in CLPD-NK, and STAT5b mutations have also been detected in T-LGLL.<sup>23–26</sup> Using a network-modeling approach, it was also found that interleukin (IL) 15 and platelet-derived growth factor (PDGF) are the two key mediators controlling interactions among these survival pathways.<sup>27</sup> In a transgenic mouse model resulting in constitutive murine IL-15 production, there is clonal expansion of LGLs that show overlapping features with human LGLL diseases.<sup>28,29</sup>

Targeting of normal tissue by leukemic LGLs may also play a role in disease pathogenesis. Lysis of endothelial cells resulting from activation of NK receptors via signaling partners DAP10 and DAP12 could explain development of pulmonary hypertension observed in some patients with LGLL.<sup>30</sup>

## HISTOLOGIC AND IMMUNOPHENOTYPIC FEATURES

The marrow biopsy in T-LGLL may be hypo-, normo-, or hypercellular, often with preserved trilineage hematopoiesis. There may be occasional nodules of reactive CD4+ and B lymphocytes as well as scattered LGLs, which are better seen in the aspirate. The presence of interstitial or intrasinusoidal clusters of at least eight CD8+ or TIA-1+ LGLs or at least six granzyme B+ LGLs has been correlated with marrow involvement by LGLL.<sup>31</sup> Various superimposed findings may reflect secondary immune diseases, such as granulocyte maturation arrest and absence of red cell precursors (red cell aplasia). T-LGLL invariably affects the spleen, where the major findings are leukemic cell infiltration of the red pulp cords and sinuses, plasma cell hyperplasia, and prominent germinal centers (Fig. 5-1).<sup>3,32</sup> Hepatic sinusoids and portal areas are infiltrated by LGLs. Lymph nodes usually are not involved but can have expanded paracortical areas containing plasma cells and LGLs.

T-LGLL and CLPD-NK share overlapping immunophenotypic features in that both often express the NK-associated markers CD16 and CD57. Aberrant expression of Nkp46 (CD335), which is normally selectively expressed by NK cells, occurs in T-LGLL.<sup>33</sup> CD56, which is constitutively expressed by circulating NK cells in healthy individuals, may be downregulated in CLPD-NK, and its expression in T-LGLL may be associated with a less favorable clinical course.<sup>34</sup> T-leukemic LGLs usually are CD3+, CD4-, CD8+, CD16+, CD56-, and CD57+ and often human leukocyte antigen (HLA) DR+. Less commonly, leukemic LGLs express CD4 with variable CD8 expression.<sup>35</sup> Leukemic T-LGL usually expresses the TCR  $\alpha\beta$  heterodimer, although cases with similar clinical features have been described that express the  $\gamma\delta$

TCR heterodimer.<sup>36</sup> In contrast to normal LGLs of T-cell origin, leukemic LGLs express significantly lower levels of CD5 and show abnormal killer immunoglobulin-like receptor (KIR) expression.<sup>37</sup> The neoplastic NK cells often demonstrate abnormal KIR expression with either complete absence of surface KIR or restricted KIR expression indicating outgrowth of a clonal population.<sup>38</sup>

## CLINICAL AND LABORATORY FEATURES AT PRESENTATION

Table 5-1 summarizes the clinical features of T-LGLL, as reported in six large published series. Men and women are equally affected, and median age at diagnosis is approximately 60 years. Only a minority of patients are less than 50 years old. Approximately one-third of patients with LGLL are asymptomatic at presentation, and the diagnosis is made on an examination of the blood. The remainder of the patients typically present with symptoms related to neutropenia (80 percent), anemia (45 percent), or both. B symptoms are present in only 15 percent of patients. Physical examination reveals mild-to-moderate splenomegaly in 35 percent and hepatomegaly in up to 20 percent. Lymphadenopathy and skin involvement are rare. Pulmonary hypertension develops in occasional cases.<sup>30</sup>

Rheumatoid arthritis is often a prominent feature of LGLL, sometimes resulting in a clinical picture that is difficult to distinguish from that of Felty syndrome (FS) (chronic arthritis, splenomegaly, and granulocytopenia in the background of long-standing seropositive rheumatoid arthritis).<sup>39</sup> Clonal proliferations of LGLs have been observed in patients with FS,<sup>39</sup> and it is likely that a significant subset of patients diagnosed with FS may in fact have T-LGLL. The distinction between

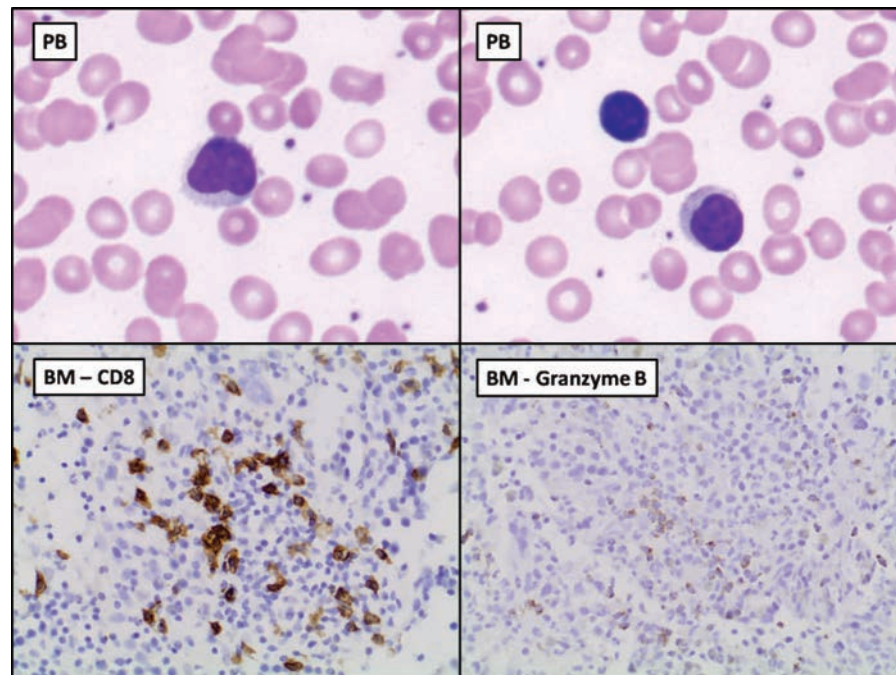


**Figure 5-1.** Morphology and immunohistochemical analysis of T-cell large granular lymphocytic leukemia (T-LGLL) in peripheral blood and bone marrow. Upper panels show high-power images of circulating large granular lymphocytes (LGLs) from a patient with T-LGLL (original magnification 500 $\times$ ). Note for comparison the small lymphocyte next to the LGL in the right panel. LGLs are slightly larger than other lymphocytes, and they have more nuclear membrane irregularity, moderate amounts of pale blue cytoplasm, and fine cytoplasmic granules. The lower panels show foci of atypical clusters of CD8+ (left) and granzyme B+ (right) lymphocytes in the BM of a patient with T-LGLL (original magnification 500 $\times$ ). The identification of atypical clusters of at least eight CD8+ or at least six granzyme B+ lymphocytes supports the diagnosis of LGLL in the appropriate clinical setting.<sup>31</sup> BM, bone marrow; PB, peripheral blood.



FS and T-LGLL in patients with rheumatoid arthritis has generally been based on whether the LGL proliferation was monoclonal (LGLL) or polyclonal (FS). However, this distinction cannot always be made, and recent cases of FS with somatic STAT3 mutations have been reported, suggesting that the two entities overlap also at the molecular level.<sup>40</sup>

The clinical presentation of CLPD-NK leukemia is similar to that of T-LGLL<sup>41</sup> and must be distinguished from that of patients with ANKL, who are younger, have systemic B symptoms, and typically have massive hepatosplenomegaly. Lymphadenopathy and gastrointestinal tract involvement are common.<sup>42</sup> Examination of the blood film is important in making the diagnosis of T-LGLL because approximately 25 percent of patients do not have an increased total lymphocyte count.<sup>13</sup> LGLs can be identified by morphology, although immunophenotyping is necessary to distinguish whether the LGLs are of T-cell or NK cell lineage (Fig. 5–2). The median LGL count of patients with T-LGLL is  $4.2 \times 10^9/L$  (Table 5–1).

Most patients (70 percent) with T-LGLL have chronic neutropenia, and approximately 35 percent have neutrophil counts less than  $0.5 \times 10^9/L$ .<sup>13</sup> Recurrent bacterial infections of the upper and lower respiratory tract are observed in 30 percent of patients. Opportunistic infections, however, are uncommon. Anemia is observed in approximately 50 percent of cases of T-LGLL. Transfusion-dependent anemia occurs in approximately 20 percent of patients. More than one mechanism of anemia has been described, including autoimmune hemolytic anemia, pure red cell aplasia, and, rarely, aplastic anemia.<sup>3,43</sup> LGLL is the most commonly associated disease in patients with pure red cell aplasia.<sup>44</sup> The role of LGLs in the suppression of erythropoiesis in a patient with LGLL was established *in vitro*,<sup>45</sup> and the role of T cells is supported by response to antithymocyte globulin.<sup>46</sup> Thrombocytopenia, usually moderate, is seen in approximately 20 percent of patients. It may be immune mediated (presence of antiplatelet antibodies), a result of splenic sequestration, and rarely secondary to amegakaryocytosis. Rare cases of amegakaryocytic thrombocytopenia or red cell aplasia have occurred and have responded to immunosuppressive therapy.<sup>3,47</sup>

## ● DIAGNOSTIC CRITERIA

While the characteristic clinical triad of blood cytopenias, clonal expansion of blood LGLs, and elevated RF titer is now well recognized and useful in clinical practice, the definition of specific diagnostic criteria for LGLL has been a subject of considerable controversy. As a consequence, patient selection criteria have been inconsistent, and inclusion rules for outcome studies and clinical trials have not been harmonized, making comparative interpretations of data a challenge. This fact likely explains the large differences in the frequency of specific clinical manifestations across LGLL studies and raises concerns about the validity of comparing outcome analyses from different centers.

In the absence of molecular hallmarks, and with a significant level of overlap in clinical and laboratory features between reactive and neoplastic LGL proliferations, the diagnosis of LGLL hinges in part on the selection of the cutoff value for blood LGL levels. Historically, a diagnosis of LGLL required the detection of  $2 \times 10^9/L$  or greater blood LGLs. However, there are patients with less than  $2 \times 10^9/L$  blood LGLs that clearly have monoclonal CD8+ T-cell lymphoproliferations and display clinical and laboratory features, as well as outcomes, indistinguishable from LGLL. This finding has led to the adoption of less restrictive criteria for the diagnosis of LGLL. Currently, the most commonly accepted blood LGL cutoff for a diagnosis of LGLL is greater than  $0.5 \times 10^9/L$ , with a TCR $\alpha\beta$ + / CD3+ / CD8+ / CD57+ immunophenotype, lasting more than 6 months.<sup>48</sup> For CLPD-NK (CD3– / CD8+ / CD16+ and/or CD16+ / CD56+) the cutoff is greater than  $0.75 \times 10^9/L$ .

## ● DIFFERENTIAL DIAGNOSIS

The diagnosis of LGLL should be considered in patients with chronic or cyclic neutropenia<sup>49</sup> and in patients with pure red cell aplasia or rheumatoid arthritis who have increased concentrations of LGLs. HIV infection can lead to a mildly increased concentration of LGL cells; however,

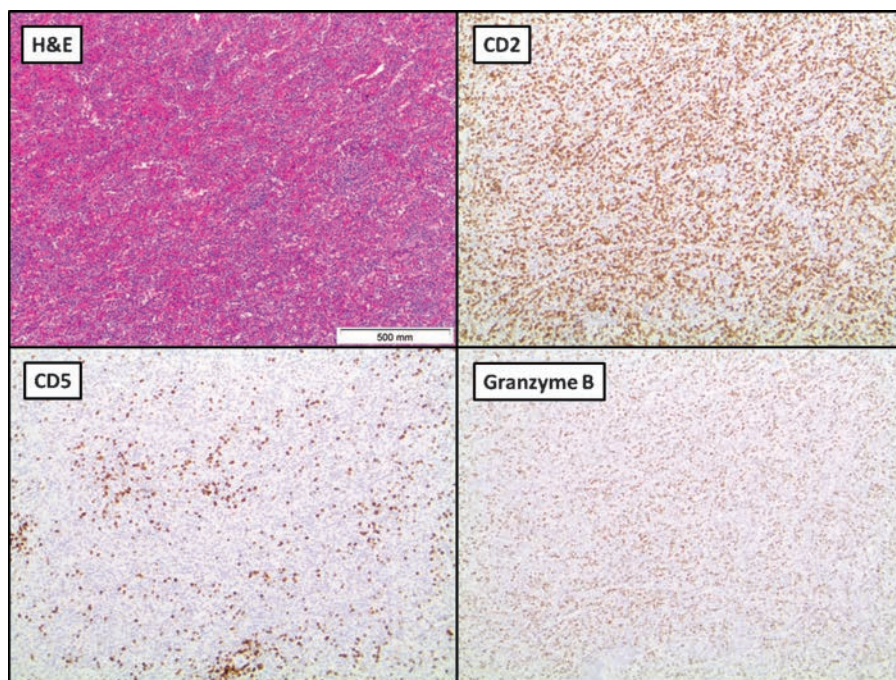


**Figure 5–2.** Morphology and immunohistochemical analysis of T-cell large granular lymphocytic leukemia (T-LGLL) in the spleen. Panels demonstrate red pulp involvement in the spleen of a patient with T-LGLL (original magnification 100 $\times$ ). In this example, lymphocytes are increased in the red pulp, which is expanded. Furthermore, by immunohistochemical analysis, the lymphocytes show an aberrant immunophenotype with expression of CD2 and granzyme B but downregulation of the pan-T-cell marker CD5. H&E, hematoxylin and eosin.



in these patients the LGLs are not monoclonal.<sup>50</sup> Reactive expansions in LGLs may also be seen in the settings of other viral infections, such as CMV, postallogeic stem cell transplantation, hyposplenism, and pharmacologic tyrosine kinase inhibition.<sup>13,51,52</sup> Molecular studies to identify a clonal *TCR* gene rearrangement may be helpful in distinguishing reactive from neoplastic LGL proliferations; however, reactive clonal T-cell LGL expansions can occur.<sup>53,54</sup> X-chromosome inactivation studies may be used to establish NK cell clonality in female patients.<sup>55</sup> The immunophenotypic features described previously may be useful to screen for an aberrant LGL population; however, reactive *TCR*γδ+ T cells can show a similar pattern of surface expression, including absence of CD4 with or without dim partial CD8 and expression of NK-associated markers such as CD56 expression.<sup>56</sup>

The differential diagnosis also includes blood, marrow, or spleen involvement by other T-cell neoplasms. Often, the morphologic features in other T-cell neoplasms are more suggestive of malignancy compared to LGL; however, this is not always the case. The clinical history is also important to consider. For example, both T-LGL and hepatosplenic T-cell lymphoma (HSTCL) (Chap. 15) show a similar tissue distribution and with overlap in morphologic features. However, HSTCL is more common in young men, whereas T-LGL typically affects older individuals without a predilection for gender. Moreover, whereas T-LGL cells express TIA-1, perforin, and granzyme B cytolytic granules, HSTCL characteristically expresses TIA-1 but lacks perforin and granzyme B expression.<sup>31,57,58</sup> Consequently, close attention to these subtle details is important in evaluating a potential T-cell lymphoproliferative disorder.

## CLINICAL COURSE, THERAPY, AND PROGNOSIS

LGLL is typically a chronic, indolent, lymphoproliferative disorder with a 5-year survival close to 90 percent. Clinical symptoms and hematologic abnormalities are often only modestly to moderately severe, and many patients can remain infection free and transfusion independent for a long time. Therefore, treatment at diagnosis is not always indicated, and, in the absence of curative strategies, the main goal of therapy for most patients is amelioration of the secondary clinical manifestations of the disease, rather than cytorreduction of leukemic LGLs in the blood and marrow. For asymptomatic patients with modest neutropenia and anemia, risk-versus-benefit analysis may not favor starting immediate treatment, and with no data supporting improved survival with early initiation of therapy, observation may be a better option. As in other chronic lymphoid neoplasms, therefore, it is important to define acceptable indications for therapy. These criteria typically include (1) severe neutropenia ( $<0.5 \times 10^9/L$ ) or moderate neutropenia ( $0.5$  to  $1.0 \times 10^9/L$ ) with recurrent infections; (2) symptomatic or transfusion-dependent anemia; (3) moderately severe thrombocytopenia ( $<50 \times 10^9/L$ ); and (4) associated autoimmune conditions requiring therapy.<sup>59</sup>

For patients who are symptomatic and exhibit one or more of the indications for treatment, the most commonly adopted approach is chronic immunosuppression with low-dose oral methotrexate (MTX; 10 to 20 mg/week), cyclophosphamide (50 to 100 mg/day), or cyclosporine (variable daily doses, with or without monitoring of serum cyclosporine levels).<sup>59,60</sup> Response rates with each of these drugs are in the approximately 50 percent range, with time to response being from 4 to 16 weeks and with acceptable and comparable safety profiles.<sup>59</sup> The optimal agent for initial therapy of LGLL is not currently known because these approaches have never been directly compared in a randomized study, and efficacy data come primarily from small, retrospective case series,

often using different response criteria.<sup>61–63</sup> In a crossover study assessing the efficacy of MTX versus cyclophosphamide in LGLL, 55 patients received MTX as initial therapy, and nonresponders were treated with cyclophosphamide.<sup>63a</sup> The response rate to MTX was 38 percent. Of the nonresponders, 64 percent responded to cyclophosphamide. A randomized trial (NCT01976182) of first-line MTX versus cyclophosphamide is in progress in France.

Prednisone (1 mg/kg) may be briefly effective as a single agent or accelerate the initial response to one of the first-line immunosuppressive agents. Glucocorticoids, however, do not have long-term efficacy in LGLL, and neutropenia generally recurs as soon as the medication is tapered. Because significant morbidity and mortality can result from chronic severe neutropenia<sup>13</sup> and transient responses to granulocyte and granulocyte-monocyte colony-stimulating factors<sup>64–66</sup> have been reported, the judicious use of myeloid growth factors may be advantageous in patients before surgical procedures or during sepsis.

Methotrexate, cyclophosphamide, or cyclosporine, with or without prednisone, are usually effective in correcting the autoimmune cytopenias and the pure red cell aplasia associated with T-LGLL.<sup>13</sup> In cases of treatment failure with the three front-line therapies, the monoclonal antibody alemtuzumab (Campath-1H), which targets CD52 expressed on the surface of T-LGLL, has been effective in case reports and small case series, including patients with refractory red cell aplasia.<sup>67</sup>

Splenectomy has been reported to produce responses in up to 50 percent of patients with T-LGLL.<sup>59</sup> However, splenectomy should be considered only for patients with resistant disease who have persistent evidence of severe cytopenia or symptomatic splenomegaly.

New therapeutic approaches, based on our understanding of the molecular pathogenesis of LGLL, are being investigated. A small phase II study of the farnesyltransferase inhibitor tipifarnib showed a significant improvement in marrow hematopoiesis and increased hematopoietic colony growth *in vitro* but no objective responses.<sup>67a</sup> Considering the frequency of gain-of-function JAK/STAT mutations in LGLL, JAK/STAT inhibitors represent a logical and attractive treatment option for LGLL. OPB-31121, a STAT3 and STAT5 inhibitor, showed antitumor activity *in vitro* and *in vivo* in preclinical models of malignancies harboring BCR-ABL, FLT3/ITD, and JAK2 V617F, which are dependent on STAT3/5 signaling.<sup>67b</sup> Another study showed hematologic responses and improvement in neutrophil counts with tofacitinib in patients with refractory LGLL and rheumatoid arthritis.<sup>67c</sup> Bortezomib demonstrated efficacy in an LGLL leukemia model in IL-15 transgenic mice<sup>67d</sup> and should also be considered in LGLL.

Patients with chronic NK lymphocytosis usually do not require treatment.

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## CHAPTER 6

# GENERAL CONSIDERATIONS OF LYMPHOMAS: EPIDEMIOLOGY, ETIOLOGY, HETEROGENEITY, AND PRIMARY EXTRANODAL DISEASE

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### SUMMARY

Lymphomas are a heterogeneous group of malignancies that originate from neoplastic transformation of a lymphocyte that has undergone mutations that confer growth and survival advantages compared to their normal cellular counterparts. These neoplasms usually originate in lymph nodes or lymphatic tissue in other sites (extranodal lymphoma) and can be localized or widespread at the time of diagnosis. Men are affected more frequently than women, and the risk of acquisition of most lymphomas increases logarithmically with age. Classification systems have considered the likely lymphoid progenitor that corresponds to the phenotype (immunotype) and genotype of the malignant cells in the transformed clone. The specific pathologic diagnosis is usually established by the appearance of the histopathology in tissue sections, the immunophenotypic profile of CD antigens expressed on affected lymphocytes, specific cytogenetic findings, especially translocations (e.g., t[11;14]), immunocytochemical

**Acronyms and Abbreviations:** ALCL, anaplastic large cell lymphoma; *ALK*, ALCL tyrosine kinase gene; ATLL, adult T-cell leukemia/lymphoma; CBC, complete blood count; CRu, complete remission unconfirmed; CT, computed tomography; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; FDG, 2-fluorodeoxyglucose; HHV8, human herpesvirus 8; HL, Hodgkin lymphoma; HTLV-1, human T-cell leukemia/lymphoma virus-1; *IFISH*, interphase fluorescence *in situ* hybridization; Ig, immunoglobulin; IWG, International Working Group; MALT, mucosa-associated lymphoid tissue; NCCN, National Comprehensive Cancer Network; NHL, non-Hodgkin lymphoma; NK, natural killer; PET, positron emission tomography; R-CHOP, rituximab-cyclophosphamide, hydroxydoxorubicin, vincristine (Oncovin), and prednisone; REAL, revised European-American classification of lymphoid neoplasm; RNA-seq, ribonucleic acid sequencing; SEER, Surveillance, Epidemiology, and End Results; TCE, trichloroethylene; TCR, T-cell receptor; WHO, World Health Organization.

markers (e.g., cyclin D<sub>1</sub>), and the specific tissue location (e.g., mucosa-associated lymphatic tissue). Although most lymphomas arise without an evident cause, *human T-cell leukemia/lymphoma virus 1* (HTLV-1), *Epstein-Barr virus*, *hepatitis C virus*, and *human herpes virus-8* infections, as well as infections with the bacteria *Helicobacter pylori* and, perhaps, *Chlamydomphila psittaci*, either are established as causal (e.g., HTLV-1) or have very strong associations with lymphoma incidence (*hepatitis C virus*), suggesting their role in causation. *HIV* is permissive by inducing severe immunodeficiency and setting the stage for an *Epstein-Barr virus*-induced or *human herpes virus-8*-induced lymphoma. These relationships may vary by geographic area. Several occupational and industrial exposures are suspected of being related to lymphoma incidence, for example, organochlorines, phenoxyacid herbicides, and others, but these associations have not been established with scientific certainty. At present, the estimated attributable risk of lymphoma from all suspected exogenous factors together is relatively small in proportion to the number of annual cases, leaving most cases arising *de novo*, that is, arising from spontaneous mutations related to stem or progenitor cell DNA replication and/or repair errors. There are wide discrepancies in the incidence of specific lymphoma subtypes in different geographic regions (e.g., follicular lymphoma is very common in the United States and very uncommon in East Asia). Primary extranodal lymphoma may involve virtually any tissue or organ. Depending on the site, important functional abnormalities may ensue (e.g., bilateral adrenal gland replacement and hypoadrenocorticism, hypothalamic–pituitary involvement and diabetes insipidus). Multidrug chemotherapy combinations in conjunction with lymphocyte-specific monoclonal antibody therapy form the foundation of current treatment paradigms for most lymphomas, though radiotherapy and surgical excision continue to play limited roles in selected circumstances, depending on the site and histopathology.

### ● DEFINITION AND HISTORY

Lymphomas are a heterogeneous group of malignancies of B cells, T cells, and, infrequently, natural killer (NK) cells that usually originate in the lymph nodes but may affect any organ of the body. Lymphoma previously was referred to as *lymphosarcoma* and its two major subtypes designated *reticulum cell sarcoma* and *giant follicular lymphoma* (Brill-Symmers disease).<sup>1–3</sup> In 1966, Rappaport<sup>4</sup> published a classification system based on the patterns of lymphoma cell growth, size, and shape; the system attempted to correlate morphology with clinical outcome. The classification proved to have some inaccuracies, such as the term *histiocytic lymphoma* to describe lymphoid tumors of large transformed lymphocytes that were not derived from the monocyte-macrophage lineage. Nonetheless, the Rappaport classification was an important milestone and became the most widely used classification in the United States. In 1974, Lukes and Collins proposed another classification system, which incorporated morphology with immunologic subtype, and it was endorsed by the Committee on Nomenclature.<sup>4</sup> Another scheme, the Kiel classification, introduced by Karl Lennert and colleagues, had been more popular in Europe.<sup>5</sup> By the 1970s, at least six classifications of lymphoma had been published, and the major ones included two in the United States, one in continental Europe, and one in the United Kingdom. There was no success in reaching a consensus classification that could be used worldwide. A National Cancer Institute study showed that there was poor reproducibility among different pathologists looking at the same slides and trying to classify the case of lymphoma using any existing scheme. In 1982, a Working Formulation

\*Kenneth A. Foon was a coauthor of this chapter for the eighth edition of *Williams Hematology*, and significant portions of that chapter have been retained.

sponsored by the National Cancer Institute attempted to reconcile the large number of competing classifications then in use.<sup>6</sup> The Working Formulation was clinically useful and gained wide popularity. It divided the specific subtypes among high-grade, intermediate-grade, and low-grade lymphomas, focusing in part on the expected rate of progression, and not just on the phenotype of the case in question. With advances in our understanding of the immune system and lymphocyte progenitor developmental sequences, and the availability of monoclonal antibodies for subtyping lymphoid cells and lymphocyte gene profiling, a new classification schema became possible based on cell type, tissue of origin, immunophenotype, and genotype.

In 1994, a revised European-American classification of lymphoid neoplasms (the REAL classification) was proposed by the International Lymphoma Study Group (Chaps. 1 and 7).<sup>7</sup> This group distinguished three major categories of lymphoid malignancies, which included B-cell, T-cell, and Hodgkin lymphoma (HL). Lymphomas were defined by morphologic, immunologic, and genetic techniques. Many of the lymphomas were associated with distinct clinical presentations, and cases that did not fit into defined entities were left unclassified. Further subclassification<sup>8</sup> divided each of the B-cell and T-cell lineages into (1) indolent lymphomas (low risk of rapid progression), (2) aggressive lymphomas (intermediate risk of progression), and (3) very aggressive lymphomas (high risk of progression). In 1995, a collaborative project of the European Association for Haematopathology and the Society for Hematopathology began to revise the REAL classification. In 2001, they published the World Health Organization (WHO) *Classification of Tumors of the Haematopoietic and Lymphoid Tissues* that is used in this chapter and represents the current worldwide consensus classification of malignancies that arise in a lymphocyte. In 2008, the WHO updated this classification (Chaps. 1 and 7).<sup>9,10</sup>

Emerging concepts in the staging, therapy, and response evaluation of lymphomas evolved in parallel with the changing nomenclature. Initial efforts to define the extent of disease involvement were undertaken primarily for HL and ultimately led to the Ann Arbor classification,<sup>11</sup> which subdivided patients' disease into four stages, each subclassified into A and B groups based on the presence of fevers greater than 38.3°C, weight loss, and drenching night sweats. The Ann Arbor system proved useful for decades and was subsequently applied also to non-Hodgkin lymphomas (NHLs). The Cotswold modification of the Ann Arbor Classification<sup>12</sup> first formally incorporated computed tomography (CT) into clinical staging paradigms and introduced the terms *X* for bulky disease and *complete remission unconfirmed* (CRu) to describe patients with a residual mass after treatment that most likely represented fibrous scar tissue. The sensitivity and accuracy of CT imaging of the abdomen rendered the "staging laparotomy" for assessment of the abdomen obsolete. The first universally

accepted response criteria for NHL were initially published in 1999 by the National Cancer Institute Working Group<sup>13</sup> and later revised in 2007 to incorporate positron emission tomography (PET), marrow immunohistochemistry, and flow cytometry for response assessment.<sup>14</sup> PET/CT imaging rendered the CRu designation obsolete in 2007 because residual radiographic abnormalities could be accurately determined to be either residual active lymphoma or posttreatment fibrosis, based on the metabolic activity of the lesions. These criteria were critically reviewed and analyzed by working groups at the 11th and 12th International Conferences on Malignant Lymphoma in Lugano, Switzerland, in 2011 and 2013 and at the Fourth International Workshops on Positron Emission Tomography in Lymphoma in 2012 in Menton, France.<sup>15,16</sup> These international workshops were attended by leading hematologists, oncologists, radiation oncologists, pathologists, radiologists, and nuclear medicine physicians, representing all major lymphoma clinical trials groups and cancer centers in North America, Europe, Japan, and Australasia. Their deliberations culminated in the publication in 2014 of improved criteria for the initial evaluation, staging, and response assessment for both HL and NHL<sup>17</sup> that are relevant for community physicians, investigator-led trials, cooperative groups, and registration trials. These new rules, known as the "Lugano Classification," depart substantially from older staging and evaluation systems as detailed further in this chapter.

## ● EPIDEMIOLOGY

In 2017, approximately 80,500 new cases of lymphoma were projected to be diagnosed and approximately 21,210 persons in the United States were expected to die of this disease.<sup>18</sup> These numbers represent 4.8 percent of the annual incidence of all cancers and 3.4 percent of annual cancer-related deaths. The age-adjusted incidence rates per 100,000 population provided by the Surveillance, Epidemiology, and End Results (SEER) Program of the United States National Cancer Institute are 24.9 for white males, 17.4 for black males, 17.2 for white females, and 11.9 for black females.<sup>19</sup> The increased risk for men is similar to that found in other countries, although the incidence of NHL in the United States is approximately threefold that of several underdeveloped countries and twofold that of several comparable industrialized countries.<sup>20</sup> The risk of developing lymphoma is less in the United States among persons of African descent in comparison to those of European descent. An exponential increase in incidence in NHL among men and women occurs with increasing age (Fig. 6-1). There are some notable exceptions to this overall trend, however, with lymphoblastic lymphoma occurring most commonly in children, Burkitt lymphoma in the 20- to 64-year-old age group, and primary mediastinal B-cell lymphoma developing at a median age of 35 years.<sup>20</sup>

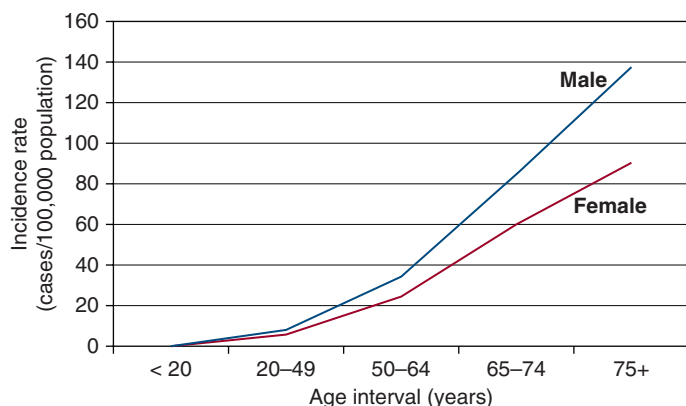


**Figure 6-1.** The graph depicts the rate of increase with age in non-Hodgkin lymphoma incidence among American males and females. This pattern is true for Americans of European or of African descent. (Data from the Surveillance, Epidemiology, and End Results [SEER] program [www.seer.cancer.gov] research data [1973–2011], National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2014, based on the November 2013 submission; 2014.)



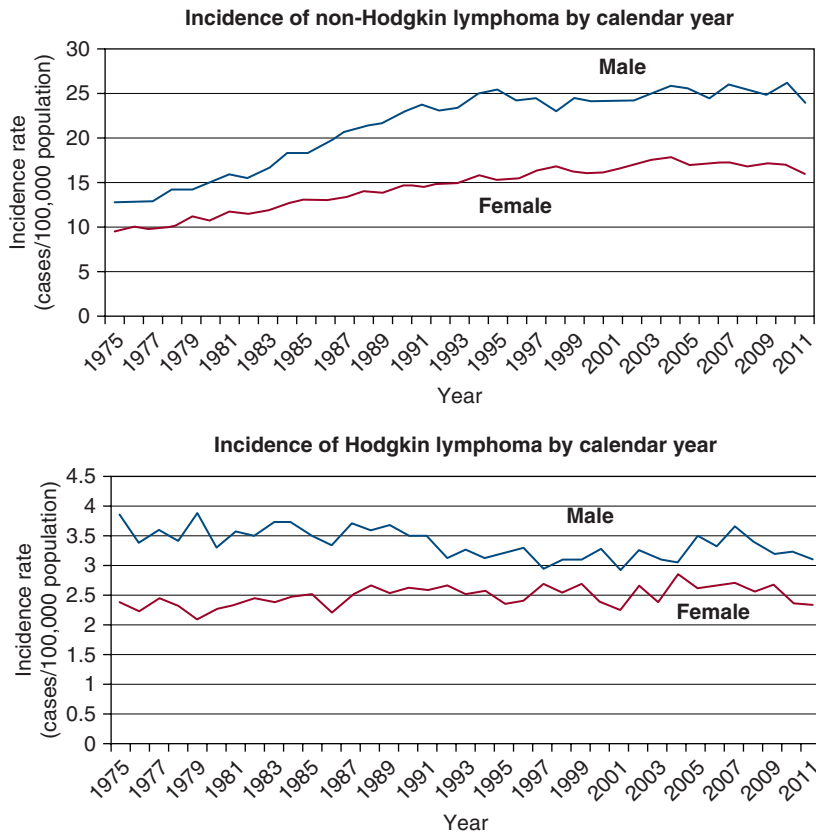


**Figure 6-2.** Incidence of non-Hodgkin and Hodgkin lymphoma by calendar year. The incidence of non-Hodgkin lymphoma approximately doubled from the early 1970s to the mid-1990s in the United States and in other industrialized countries that tracked incidence of specific cancers. No satisfactory explanation has been uncovered for this change. The “epidemic” of lymphoma ended in the mid-1990s, and the incidence curves have been “flat” since 1996. The increase in incidence was present in Americans of European and African descent and among men and women. In stark contrast and serving as an internal control, the incidence of Hodgkin lymphoma was essentially unchanged over that period of time. (Data from the Surveillance, Epidemiology, and End Results [SEER] program [www.seer.cancer.gov] research data [1973–2011], National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2014, based on the November 2013 submission; 2014.)

Follicular lymphoma represents approximately 25 percent of NHL cases in the United States but is very uncommon in many developing countries and in Asia, especially in Japan and China.<sup>21,22</sup> The United States has a higher incidence of all lymphomas than does Japan, whereas the incidence of extranodal lymphoma is higher in Japan.<sup>21,22</sup> Burkitt lymphoma occurs most frequently in sub-Saharan Africa, whereas T-cell leukemia/lymphoma is most common in southwest Japan, the southeastern United States, northeastern South America, and the Caribbean basin.

The incidence of NHL increased dramatically in the last half of the 20th century in Europe, Asia, and the United States.<sup>20–22</sup> From 1973 to 1990, inclusive, the increase in the United States was slightly more than 80 percent, or approximately 4 to 5 percent per year (Fig. 6-2). The increase in incidence started after World War II, but the best data in the United States were acquired after 1972. The increase affected men and women, all age groups except children, and most histologic types examined, though the greatest increase was in diffuse large B-cell lymphoma (DLBCL). The increased incidence per year reached a plateau in the early 1990s, except among women and older men, for whom the incidence continued to rise.<sup>21</sup> Hardell has hypothesized that new chemicals synthesized during and after World War II accounted for increased incidence trends, with subsequent improvements in safety gear and regulatory restrictions resulting in stabilization in the last decade.<sup>23</sup> HIV was not prevalent in the human population when the increase first became apparent, although in later years HIV-related lymphoma may have played a part in increasing incidence rates. Orbital adnexal lymphoma and mantle cell lymphoma are exceptions to the recent plateau, with each still increasing at approximately 6 percent a year.<sup>24,25</sup>

Several occupations and industries and several potentially hazardous exposures, such as pesticides, herbicides, dyes, engine exhausts, and

solvents, have been found in one study or another to be more frequent in lymphoma patients than “matched” healthy comparison groups.<sup>21,26,27</sup> The results often have been inconsistent from study to study. Expert opinion indicates that no workplace exposure has been conclusively linked to lymphoma,<sup>28</sup> although farming or living in a community in which farming is prevalent has been a frequent association with higher lymphoma incidence.<sup>21,26,27</sup>

Many publications have reported a slightly increased familial predisposition to the development of NHL, with an odds ratio of 1.5 in first-degree relatives of patients with NHL in a pooled analysis of 17 case-control studies.<sup>20,29–31</sup> Nonsyndromic familial lymphoma refers to apparently healthy family members, unlike syndromic familial lymphoma, in which immunodeficiency syndromes are the predisposing phenotype (e.g., Wiskott-Aldrich syndrome; see “Immunosuppression” section). The familial cases occur in different generations and among enough family members to strongly suggest that a predisposing unidentified gene results in an incidence above that in the population at large. Li-Fraumeni syndrome is such an example, involving germline mutations in *p53*. Alternatively, it is possible that family members inherit a susceptibility to an unidentified environmental lymphomagen.

## ETIOLOGY AND PATHOGENESIS

### HISTOPATHOLOGIC HETEROGENEITY

Unlike other cancers, the malignancy referred to as “lymphoma” consists of nearly 80 phenotypes.<sup>9</sup> Because each subtype of lymphoma exhibits unique nuances in its natural history, therapeutic considerations, and prognosis, unequivocal establishment of the precise subtype of lymphoma is of paramount importance in a newly diagnosed patient.

The single most important test in a lymphoma patient is an adequate biopsy of affected tissue. In most cases, this should be an excisional lymph node biopsy or generous incisional biopsy of an extranodal site. When the only sites of disease involvement are deep in the thorax or pelvis, rendering excisional node biopsy difficult, a core needle biopsy obtained with the assistance of ultrasound or CT guidance may be adequate. Fine-needle aspiration alone should never be used as the sole method of establishing the initial diagnosis of lymphoma<sup>17,32,33</sup> because the precise subtyping of lymphomas requires examination of tissue architecture, not merely cytologic examination of isolated cells. Biopsies should be subjected to microscopic examination, flow cytometry of fresh cells, immunohistochemical examination of fixed tissue sections, and cytogenetic/interphase fluorescence *in situ* hybridization (iFISH) analysis.<sup>33</sup> In the near future, it is likely that genomic analyses using ribonucleic acid sequencing (RNA-seq) technology will also become important to define specific mutations permitting personalized selection of targeted agents capable of inhibiting deranged intracellular pathways.<sup>34</sup>

Table 6-1 lists the most prevalent phenotypes distinguishable by pathologists according to the current WHO classification system.<sup>9,10</sup> Approximately 88 percent of lymphomas originate in a cell that exhibits features most consistent with B lymphocyte derivation (B-cell CD surface antigens or immunoglobulin [Ig] gene rearrangement). The remaining cases are lymphomas in which the phenotype and genotype are most closely related to T cells or NK cells (T-cell receptor [TCR] chain rearrangements or specific immunophenotypes). This heterogeneity is problematic for histopathologic diagnosis, classifying patients in clinical trials, and selecting therapy. It also makes studies of epidemiology and etiology more difficult. To understand acquired (environmental) or genetic causes of the type of lymphoma in question, the latter studies, to be insightful, must stratify the study group by specific histopathologic diagnosis. This requirement can be difficult if one is studying uncommon phenotypes.

The histopathologic diversity of lymphoma is the result of the complexity of the immune system; its wide distribution through many organs with highly specialized sites, such as mucosa-associated lymphoid tissue (MALT); its differentiation into T-lymphocyte, B-lymphocyte, and NK-lymphocytic lineages; its complex maturation through many progenitor cell levels; the concomitant sequential alterations in expression of immune complex genes and the myriad opportunities for transforming mutations; and the transforming effects of mutations in the many genes encoding Ig chains or the TCR. Thus, studies in prevalent subtypes of lymphoma such as follicular and DLBCL are more common than in uncommon or rare subtypes. In addition, epidemiologic or etiologic findings relevant to one subtype may not be relevant to another subtype or to lymphoma in general.

## ENVIRONMENTAL FACTORS

An increased incidence of lymphoma has been observed, especially among farmers and gardeners but also in printers, woodworkers, dry cleaners, barbers and hairdressers, possibly from organic solvent exposure, especially to trichloroethylene (TCE), a solvent used in many industries.<sup>20–22,26–28</sup> The increased incidence in agricultural workers may be attributed to exposure to a variety of agents, including organochlorines, organophosphates, and phenoxyacid herbicides.<sup>21,22</sup> Despite many studies, an association with lymphoma incidence with herbicide or pesticide exposure has not reached the level of scientific certainty.<sup>21</sup> Indeed, evidence indicates that modest exposure to herbicides by adults who use such products in and around their homes is not likely to increase lymphoma risk,<sup>35</sup> although heavy occupational exposures remain under study. Large studies of two pesticides, chlorpyrifos<sup>36</sup> and glyphosate,<sup>37</sup> showed no association with lymphoma incidence. In the

**TABLE 6-1. Histologic Subtypes and Relative Frequency of the Non-Hodgkin Lymphomas\***

|                                                                                                                                                     |                                                                                                              |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| A. B-cell lymphomas (~88% of all non-Hodgkin lymphoma [NHL])                                                                                        |                                                                                                              |
| 1. Diffuse large B-cell lymphomas (30%)                                                                                                             | T-cell-rich large B-cell lymphoma                                                                            |
|                                                                                                                                                     | Primary diffuse large B-cell lymphoma of the central nervous system                                          |
|                                                                                                                                                     | Primary cutaneous diffuse large B-cell lymphoma                                                              |
|                                                                                                                                                     | Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma of the elderly                               |
|                                                                                                                                                     | Diffuse large B-cell lymphoma arising in human herpesvirus (HHV)-8-associated multicentric Castleman disease |
|                                                                                                                                                     | Diffuse large B-cell lymphomas with features simulating Hodgkin lymphoma                                     |
| 2. Follicular lymphoma (25%)                                                                                                                        |                                                                                                              |
| 3. Extranodal marginal zone lymphoma of mucosa-associated lymphatic tissue (MALT lymphoma) (7%)                                                     |                                                                                                              |
| 4. Small lymphocytic lymphoma-chronic lymphocytic leukemia (7%)                                                                                     |                                                                                                              |
| 5. Mantle cell lymphoma (5%)                                                                                                                        |                                                                                                              |
| 6. Primary mediastinal (thymic) large B-cell lymphoma (3%)                                                                                          |                                                                                                              |
| 7. Lymphoplasmacytic lymphoma-Waldenström macroglobulinemia (<2%)                                                                                   |                                                                                                              |
| 8. Nodal marginal zone B-cell lymphoma (<1.5%)                                                                                                      |                                                                                                              |
| 9. Splenic marginal zone lymphoma (<1%)                                                                                                             |                                                                                                              |
| 10. Extranodal marginal zone B-cell lymphoma (<1%)                                                                                                  |                                                                                                              |
| 11. Intravascular large B-cell lymphoma (<1%)                                                                                                       |                                                                                                              |
| 12. Primary effusion lymphoma (<1%)                                                                                                                 |                                                                                                              |
| 13. Primary cutaneous follicle center lymphoma (1%)                                                                                                 |                                                                                                              |
| 14. Burkitt lymphoma-Burkitt leukemia (1.5%)                                                                                                        |                                                                                                              |
| 15. Plasmablastic lymphoma (<1%)                                                                                                                    |                                                                                                              |
| 16. Lymphomatoid granulomatosis (<1%)                                                                                                               |                                                                                                              |
| B. T- and natural killer (NK)-cell lymphomas (~12% of all NHLs)                                                                                     |                                                                                                              |
| 1. Extranodal T- or NK cell lymphoma                                                                                                                |                                                                                                              |
| 2. Enteropathy-associated T-cell lymphoma                                                                                                           |                                                                                                              |
| 3. Hepatosplenic T-cell lymphoma                                                                                                                    |                                                                                                              |
| 4. Subcutaneous panniculitis-like T-cell lymphoma                                                                                                   |                                                                                                              |
| 5. Cutaneous T-cell lymphoma (Sézary syndrome and mycosis fungoides)                                                                                |                                                                                                              |
| 6. Primary cutaneous $\gamma\delta$ T-cell lymphoma                                                                                                 |                                                                                                              |
| 7. Anaplastic large cell lymphoma                                                                                                                   |                                                                                                              |
| 8. Angioimmunoblastic T-cell lymphoma                                                                                                               |                                                                                                              |
| 9. Primary T-cell lymphoma unspecified                                                                                                              |                                                                                                              |
| C. Immunodeficiency-associated lymphoproliferative disorders (see Table 6-2 for inherited diseases associated with immunodeficiencies and lymphoma) |                                                                                                              |
| 1. HIV-associated lymphoma                                                                                                                          |                                                                                                              |
| 2. Posttransplantation lymphoproliferative disorder                                                                                                 |                                                                                                              |
| 3. Lymphoma associated with a primary immune disorder                                                                                               |                                                                                                              |

\*The parenthetical percentages are approximate but give some sense of the relative distribution of subtypes. The frequency of lymphoma varies depending on the geographic area under consideration. The frequencies cited here are approximate and related to those observed in the United States, the United Kingdom, or Western Europe. Rare subtypes are not listed (see Chap. 1, Table 1-1).

Data from Swerdlow SH, Campo E, Harris NL, et al: *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th ed. International Agency for Research on Cancer, Lyon, 2008.

former case, the relative risk of death from all causes, from any cancer, or from lymphoma was significantly less in the 22,000 applicators exposed to chlorpyrifos than in the 33,000 applicators not so exposed. It is possible that subsets of individuals may be susceptible to different exposures based on genetic differences (see “Interaction of Environment and Genotype”). Indeed, preliminary studies using single nucleotide polymorphism–based analysis have linked lymphomagenesis with normal variations (polymorphisms) in genes involved in apoptosis, cell cycle regulation, lymphocyte development, and inflammation.<sup>38–40</sup> In some cases, polymorphic genes have been associated with specific morphologic subsets of lymphoma.<sup>41</sup> Dark hair dyes have also been associated with a moderately increased risk of follicular lymphoma in women.<sup>42</sup> A meta-analysis has demonstrated a dose-response relationship of NHL with cigarette smoking, with increasing NHL risk in heavy or long-duration smokers, possibly from a suppressive effect of smoking on the immune system.<sup>20</sup> Increased body mass index is associated with an increased risk of many cancers,<sup>43,44</sup> including lymphoma.<sup>44–47</sup> Indexes above 30 to 35 kg/m<sup>2</sup> (normal = 8.5 to 25 kg/m<sup>2</sup>) are associated with an increased risk of lymphoma and a worse outcome after treatment. The risk factors discussed in this section have not been found uniformly in all studies of their association with lymphoma and should be considered provisional associations. In addition, as larger populations of patients with lymphoma, stratified by histologic type, are studied, it may become possible to dissect out etiologic relationships of exogenous exposures with specific subtypes of lymphoma but not others.

Small but significant increases in lymphoma are associated with radiation exposure. An increased incidence of lymphoma was reported in survivors of the atomic bombings in Hiroshima and Nagasaki who were near the hypocenter of the detonation.<sup>48–51</sup> An increased incidence of lymphomas also has been reported for individuals at the Chernobyl accident site who received radiation equivalent to that of the atomic bomb exposures in Japan.<sup>52</sup> Individuals treated a half century ago with radiation for ankylosing spondylitis also had a small increase in lymphoma incidence.<sup>53</sup> The relative risk of lymphoma with high-dose radiation is low, and some still question the causal relationship.<sup>54</sup> Several studies have found an inverse relationship between an individual's exposure to ultraviolet light and lymphoma incidence (especially DLBCL).<sup>55</sup>

## INTERACTION OF ENVIRONMENT AND GENOTYPE

Polymorphic immune gene variations have been identified as significant factors in the association of organochloride exposure with increased incidence of lymphoma in a large population of patients and matched controls.<sup>56</sup> Associations between all exposures and NHL risk were limited to the same genotypes for interferon- $\gamma$ , *IFNG* (C-1615T) TT, and interleukin-4, *IL4* (5'-UTR, Ex1-168C→T) CC. Associations between PCB180 in plasma and dust and NHL risk were limited to the same genotypes for interleukin-16, *IL16* (3'-UTR, Ex22+871A→G) AA; interleukin-8, *IL8* (T-251A) TT; and interleukin-10, *IL10* (A-1082G) AG/GG. This result indicates that the relation between organochlorine exposure and NHL risk may be modified by particular variants in immune genes, supporting the concept of a gene-environment interaction for induction of NHL.

## INFECTIOUS AGENTS

### Human T-Cell Leukemia/Lymphoma Virus-1

Adult T-cell leukemia/lymphoma (ATLL) provides the most compelling evidence for a viral etiology of lymphoma.<sup>57</sup> Human T-cell leukemia/lymphoma virus-1 (HTLV-1) is a C-type RNA tumor virus,

isolated from patients with ATLL.<sup>58</sup> HTLV-1 is an acquired retrovirus that is not related to other known animal retroviruses. HTLV-1 can immortalize lymphoid cells in culture and induce malignancy in an infected human host. The incidence of infection with HTLV-1 in endemic areas is very high, yet few of these infected patients develop ATLL. HTLV-1 also leads to a neurologic disorder called *tropical spastic paraparesis*.<sup>59</sup> Host determinants affect transformation of lymphocytes by HTLV-1, and these may be genetic factors.<sup>58</sup> Development of ATLL is associated with infection by the virus.<sup>60</sup> Serum specimens from Japanese patients with ATLL are positive for HTLV-1, as are serum samples from ATLL patients in the Caribbean, where ATLL is endemic.<sup>61</sup> The highest prevalence of ATLL in Japan is in the southern island of Kyushi, where 10 to 15 percent of the population has antibody to HTLV-1.<sup>62</sup> On the Japanese islands where ATLL is rare, the rate is less than 1 percent. These and additional data from the Caribbean, the southeastern United States, South America, and Africa indicate that ATLL clusters in regions where HTLV-1 is prevalent.<sup>60,61</sup> How these regions are linked is not known. One hypothesis is that HTLV-1 was brought to the Americas from Africa by the slave trade and then to the southern islands of Japan by trade between Japan and Africa.<sup>61,63</sup>

Host susceptibility, a shared environmental exposure, or both contribute to HTLV-1 infection. The prevalence of HTLV-1 antibodies in close family members is three to four times higher than in the corresponding normal population.<sup>63,64</sup> In some instances, cell cultures of antibody-positive, clinically normal patients yield HTLV-1 isolates.<sup>64</sup> Blood donors are routinely screened for antibodies to HTLV-1 to prevent transmission by this route.

### Epstein-Barr Virus

Some B-cell lymphomas, including Burkitt lymphoma, posttransplantation lymphoma, and HIV-associated lymphomas (immunodeficiency-related Burkitt lymphoma, primary CNS lymphoma, primary effusion lymphoma, the immunoblastic-plasmacytoid type of DLBCL, and oral cavity plasmablastic lymphoma) may be caused by Epstein-Barr virus (EBV; Chaps. 9 and 13).<sup>65</sup> EBV is a DNA virus in the herpesvirus family that first was described in cultured lymphoblasts from patients with African Burkitt lymphoma.<sup>66</sup> EBV binds to the CD21 antigen (also the receptor for the C3d component of complement) on B lymphocytes.<sup>67</sup> It is capable of transforming B lymphocytes into lymphoblastoid cells that may proliferate perpetually in cell culture.<sup>68</sup> EBV is present in greater than 6 percent of cases of endemic Burkitt lymphoma and in approximately 20 percent of cases of nonendemic Burkitt lymphoma.<sup>69,70</sup> Malaria is holoendemic in regions where endemic Burkitt lymphoma exists.<sup>71</sup> A three-step process in the development of this lymphoma has been proposed<sup>72,73</sup>: (1) EBV initiates a polyclonal proliferation of B cells; (2) malaria or other infections further stimulate the proliferating B cells; and (3) the transforming B cells incur specific reciprocal translocations of chromosome 8 with chromosome 2, 14, or 22, resulting in a clonal expansion of B lymphocytes.

Extranodal NK/T-cell lymphoma, nasal type, is mostly endemic to East Asia and is usually associated with EBV infection (Chap. 15). The EBV genome is typically detected in the lymphoma cells.<sup>74,75</sup> Geographic localization of extranodal NK/T-cell lymphoma matches the endemic distribution of EBV, suggesting the role of EBV in lymphomagenesis.

### Human Herpesvirus-8

Human herpesvirus-8 (HHV-8) is associated with Kaposi sarcoma, Castleman disease, and primary effusion lymphoma, found most commonly in immunodeficient individuals infected with HIV.<sup>65,76–79</sup> HHV-8 is not a ubiquitous virus. It is mainly endemic in areas where classical or Kaposi sarcoma is of high prevalence, including the



Mediterranean basin and East and Central Africa. In the last areas, the HHV-8 seroprevalence can reach 80 percent in the adult population.<sup>79</sup> In the homosexual population (mainly in the United States and Europe), HHV-8 is principally transmitted during repeated sexual contacts, whereas in Africa it is mainly transmitted from mother to child and among siblings. Saliva seems to play a major role in HHV-8 transmission.<sup>79</sup> Posttransplantation primary effusion lymphoma is associated with HHV-8.<sup>76</sup>

### Hepatitis Viruses B and C

Hepatitis B and C have been implicated in the pathogenesis of lymphoproliferative diseases.<sup>80</sup> In one study, 334 newly diagnosed lymphoma patients and 1014 controls had a serologic evaluation for the presence of prior hepatitis virus B or C infection.<sup>81</sup> The results suggested that hepatitis B seropositivity was significantly higher in patients with DLBCL and follicular lymphoma, and seropositivity for hepatitis C was significantly higher in DLBCL patients. A similar result was found in another study in Taiwan, an area with a high frequency of hepatitis B virus infection.<sup>82</sup> In two other studies, seropositivity for hepatitis C was significantly higher in patients with B-cell lymphomas.<sup>83,84</sup> Hepatitis C virus has a predilection for B cells. Hepatitis C virus RNA levels are significantly higher in B cells than CD4+ or CD8+ T cells or other cells in infected patients, and the virus is associated with immunopathologic reactions, such as cryoglobulinemia, and, not infrequently, clonality of infected B lymphocytes.<sup>85</sup> Hepatitis C virus infection may be associated with the onset of DLBCL, marginal zone lymphoma, and lymphoplasmacytic lymphoma, but not follicular lymphoma.<sup>83</sup>

### *Helicobacter pylori*

*Helicobacter pylori* can cause marginal zone B-cell MALT lymphomas of the stomach and probably causes some of the higher grade lymphomas, either from transformation of a MALT or *de novo* large cell lymphoma.<sup>86–88</sup> This spiral gram-negative bacillus is the first bacterium demonstrated to cause a human neoplasm. It had been thought that the stomach was sterile because of the acid environment, but *H. pylori* had evolved to tolerate the environment, perhaps in part because it secretes urease, an enzyme that converts urea to ammonia, making the microenvironment around the organism less acidic. Although the stomach has no endogenous lymphoid tissue, the latter develops in response to the organism, and ultimately the chronic inflammatory reaction can result in the transformation and selection of a mutant lymphocyte with a growth and survival advantage, leading to a lymphoma (Chap. 12). Eradication of *H. pylori* with antibiotics early in the course of gastric MALT lymphoma can lead to regression of the lymphoma and permanent cure of the majority of afflicted patients.<sup>89</sup>

### *Chlamydia psittaci*

Ocular adnexal lymphomas are the most common tumor of the eye.<sup>90,91</sup> The majority of ocular adnexal lymphomas are extranodal, MALT lymphomas and have been linked to *Chlamydia psittaci* infection in several reports. In one study, this organism was detected in lymphoma tissue in 75 percent of cases.<sup>92</sup> DNA was detected in conjunctival swabs and/or blood mononuclear cells from 50 percent of patients. Mononuclear phagocytes were the carriers of *C. psittaci* in this population of patients.<sup>93</sup> Confirmatory reports of the association of *C. psittaci* with adnexal ocular lymphoma have been published,<sup>94,95</sup> but other studies reported no association.<sup>96–98</sup> These discrepant reports may be explained by the association of different organisms with ocular lymphoma in different geographic regions of the world.<sup>99,100</sup>

### Other Bacteria

Other bacterial infestations have been found in association with lymphomas of MALT. *Campylobacter jejuni* and *Borrelia burgdorferi* have been connected to the onset of immunoproliferative disease of the small intestine and B-cell lymphoma of the skin.<sup>101</sup>

## IMMUNOSUPPRESSION

### Inherited

A number of the rare immunodeficiency syndromes tabulated in Table 6–2<sup>82,102–123</sup> result from gene mutations leading to deficiencies in cellular or humoral immunity or both. These syndromes have not only a paradoxically high frequency of autoantibodies but also, more relevant to this discussion, an increased probability of developing a lymphoma. Because these syndromes are so uncommon, reliable assessment of the increased risk of lymphoma often has to be inferred. The increased risk to approximately 0.5 to 10 percent of patients, depending on the immunodeficiency disease in question, is several orders of magnitude above the risk in the general population (age-adjusted incidence rate in the United States younger than 65 years of age: males = 0.011 percent and females = 0.008 percent). With the exception of common variable immunodeficiency and the syndromes present in childhood and because several are X-chromosome linked, males are affected more commonly than females. The lymphomas induced may result from a susceptibility to EBV, and the lymphoproliferation may initially be polyclonal before evolving to a monoclonal tumor. Extranodal involvement appears to be more common than in persons with lymphoma who are immunocompetent. The initial manifestations of the immunodeficiency are usually infections or autoimmune abnormalities, such as immune cytopenias, and lymphoma is a later complication. We have included Li-Fraumeni syndrome in this cluster for convenience of presentation. This germline predisposition syndrome does not have an immunodeficiency phenotype as do all the other entries in Table 6–2. Rather, it is a nonsyndromic familial cancer syndrome transmitting susceptibility to mutations in *p53*. The cancers that occur in these families include lymphoma.

### Acquired

A variety of types of immunosuppressed individuals develop lymphoma, including AIDS-related lymphoma.<sup>124</sup> Posttransplantation lymphoproliferative diseases generally display B-cell lineage derivation, involvement of extranodal sites, aggressive histology and clinical behavior, and frequent association with EBV infection. The occurrence of Ig V mutations in the overwhelming majority of posttransplantation lymphoproliferative disease indicates that malignant transformation targets germinal center B cells and their descendants, in both EBV-positive and EBV-negative cases.<sup>125–127</sup> Posttransplantation T-cell lymphomas may occur and often arise in extranodal sites, such as skin or CNS.<sup>128,129</sup>

The incidence and severity of lymphomas have increased with the introduction of immunosuppressive agents such as cyclosporine, infliximab, and etanercept for treatment of autoimmune diseases.

## AUTOIMMUNITY

Several autoimmune disorders are risk factors for lymphoma, probably as a result of chronic immune stimulation, leading to excessive B-cell proliferation as well as depressed regulatory T-cell function. The strongest associations are for primary Sjögren syndrome, systemic lupus erythematosus, and rheumatoid arthritis, with relative risks of 18.8, 7.4, and 3.9, respectively.<sup>20</sup> Patients with Sjögren syndrome are especially susceptible to development of parotid gland marginal zone lymphoma (1000-fold increased risk), DLBCL, and follicular lymphomas

**TABLE 6-2.** Inherited Syndromes Predisposing to Lymphoma

| Altered Genes                                                            |             |                                                                |                                                                                                      |                                                          |               |
|--------------------------------------------------------------------------|-------------|----------------------------------------------------------------|------------------------------------------------------------------------------------------------------|----------------------------------------------------------|---------------|
| Syndrome                                                                 | Inheritance | Description                                                    | Mechanism                                                                                            | Leukemia Type                                            | References    |
| <b>DNA REPAIR DEFECTS</b>                                                |             |                                                                |                                                                                                      |                                                          |               |
| Ataxia telangiectasia                                                    | R           | <i>ATM</i> homozygotes<br>Dominant-negative missense mutations | Genomic instability<br>Increased translocations in T cells formed at the time of V(D)J recombination | T-cell lymphoma, T-cell ALL, T-cell PLL, B-cell lymphoma | 112, 115      |
| Bloom                                                                    | R           | <i>BLM</i>                                                     | Genomic instability                                                                                  | ALL, lymphoma                                            | 107, 108      |
| Nijmegen breakage                                                        | R           | <i>NBS1</i>                                                    | Genomic instability<br>Altered telomere maintenance                                                  | Lymphoid tumors, especially B-cell lymphoma              | 103, 109      |
| <b>TUMOR-SUPPRESSOR GENE DEFECT</b>                                      |             |                                                                |                                                                                                      |                                                          |               |
| Li-Fraumeni*                                                             | D           | <i>p53</i>                                                     | Defect in tumor suppressor                                                                           | CLL, ALL, Hodgkin and Burkitt lymphoma                   | 111, 120      |
| <b>IMMUNODEFICIENCY STATES</b>                                           |             |                                                                |                                                                                                      |                                                          |               |
| Common variable immunodeficiency                                         | R and D     | Defect in CD40 signaling                                       | Failure of B-cell maturation                                                                         | Burkitt, MALT, other B-cell lymphomas, Hodgkin lymphoma  | 102, 240      |
| Severe combined immunodeficiency disease (SCID)                          | R           | <i>ADA</i>                                                     | Defective T-cell + B-cell function                                                                   | B-cell lymphoma                                          | 113           |
| Wiskott-Aldrich                                                          | X           | <i>WASP</i>                                                    | Signaling and apoptosis                                                                              | Hodgkin and non-Hodgkin lymphoma                         | 117, 119      |
| X-linked immunodeficiency with normal or increased immunoglobulin (Ig) M | X           | <i>CD40L</i>                                                   | CD40 ligand defect on T cell                                                                         | Hodgkin and non-Hodgkin lymphoma                         | 116, 123      |
| X-linked lymphoproliferative (XLP) syndrome                              | X           | <i>SAP</i>                                                     | Defect in immune signaling                                                                           | EBV-related B-cell lymphoma                              | 110           |
| <b>APOPTOTIC DEFECT</b>                                                  |             |                                                                |                                                                                                      |                                                          |               |
| Autoimmune lymphoproliferative syndrome (ALPS)                           | D           | <i>APT (FAS)</i>                                               | Germline heterozygous <i>FAS</i> mutations; defective apoptosis                                      | Lymphoma                                                 | 106, 241      |
| <b>UNKNOWN DEFECT</b>                                                    |             |                                                                |                                                                                                      |                                                          |               |
| Dubowitz                                                                 | R           | Unknown                                                        | Unknown                                                                                              | ALL, lymphoma                                            | 105           |
| Poland                                                                   | D           | May not be inherited                                           | Unknown                                                                                              | ALL, lymphoma                                            | 104, 114, 118 |
| Wilms tumor (WT)                                                         | D           | Unknown                                                        | Unknown                                                                                              | ALL, Castleman disease                                   | 122           |

ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; D, dominant; EBV, Epstein-Barr virus; MALT, mucosa-associated lymphoid tissue lymphoma; R, recessive; T-PLL, T prolymphocytic leukemia; V(D)J, variable diversity joining; X, X linked.

\*Li-Fraumeni or Li-Fraumeni-like syndrome has been described in which a gene other than *p53* is mutated. *hCHK2* in particular has been described as etiologic.<sup>242,243</sup> We have not included these variants in the table because we are uncertain if lymphoma is one of the cancers for which susceptibility is increased.

Data from Segel GB, Lichtman MA: Familial (inherited) leukemia, lymphoma, and myeloma: An overview. *Blood Cells Mol Dis* 2004 Jan-Feb;32(1):246-261.

(Fig. 6-3). Similarly, marginal zone lymphoma and DLBCL are the subtypes most likely to develop in patients with lupus.<sup>130</sup> DLBCL has also been reported in association with autoimmune hemolytic anemia. The risk of developing a T-cell lymphoma is increased for patients with celiac disease and psoriasis. Hashimoto thyroiditis not only is associated with an increased risk of thyroid marginal zone lymphoma but also may have a higher risk of marginal zone lymphoma in other locations.<sup>131</sup> Sarcoidosis is an inflammatory (granulomatous) disorder that may predispose to lymphoma.<sup>132</sup>

## ● CHROMOSOMAL TRANSLOCATIONS ASSOCIATED WITH HISTOPATHOLOGIC SUBTYPE

Chromosomal abnormalities involving all chromosomes may occur in lymphomas. Lymphoid malignancies have a high frequency of translocation-inducing fusion genes. Usually, they are of two types: one involving oncogenes that are activated by juxtaposition with *IgH*

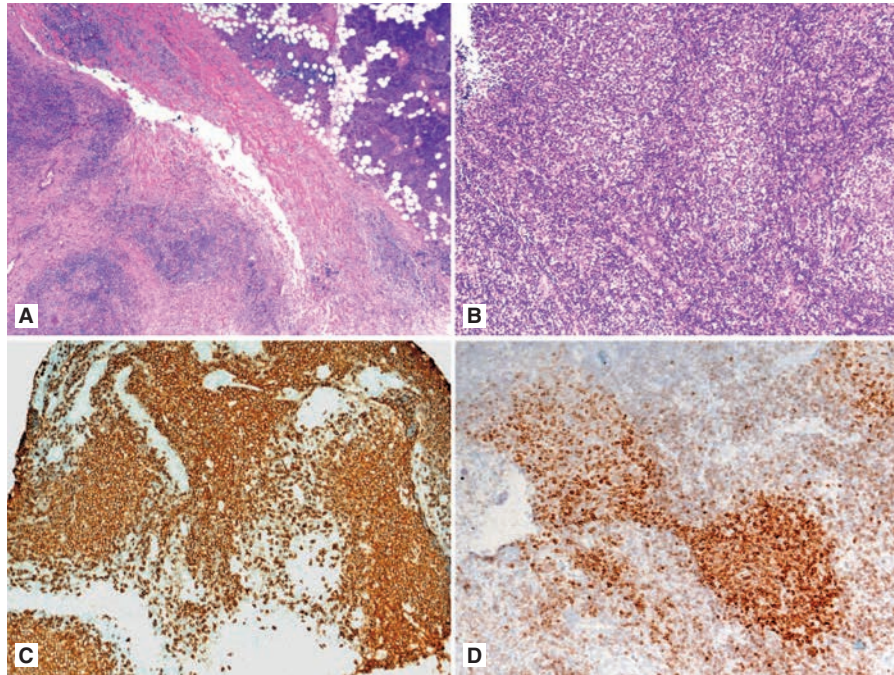


**Figure 6-3.** Primary extranodal follicular lymphoma in a parotid gland of a 67-year-old woman. **A.** Upper right corner shows normal salivary gland tissue adjacent to a lymphomatous follicular infiltrate with intense fibrosis. **B.** Lymphoma infiltrate with a vaguely nodular appearance. **C.** Nodular appearance better appreciated after immunohistochemical staining for the B-cell marker CD20. **D.** Lymphomatous follicles display overexpression for BCL-2, which would not be seen in reactive germinal centers. Flow cytometric evaluation showed a monotypic CD10+ B-cell population with  $\lambda$  light-chain restriction and BCL-2 expression on cytoplasmic staining (not shown). (Used with permission from Raymond Felgar, University of Pittsburgh Medical Center.)

or *TCR* genes or the other by formation of chimeric genes that constitutively activate mutant kinases or mutant transcription factors. The molecular alterations leading to translocations involving nonimmune genes are not known, whereas there is strong evidence that mistakes in V(D)J (variable diversity joining) recombinase activity are responsible for those translocations involving *IgH* or the *TCR* genes.<sup>133</sup>

As in translocations associated with childhood acute lymphocytic leukemia and adult chronic myelogenous leukemia (CML) and acute myelogenous leukemia, translocations involving *t*(14;18)(*IgH*; *BCL-2*) are found in healthy persons. Presumably, additional genetic events are required for transformation of a lymphocyte to occur. Alternatively, cells containing these translocations may be entering an apoptotic process, destined to be eliminated.<sup>133</sup>

## FOLLICULAR LYMPHOMA

Approximately 85 percent of follicular lymphomas carry the chromosomal translocation *t*(14;18)(q32;q21) in which the *BCL-2* oncogene on chromosome 18q21 is brought in continuity with the *Ig* heavy-chain loci on 14q32,<sup>134</sup> resulting in overexpression of the BCL-2 protein.<sup>135</sup> The accumulation of the BCL-2 protein permits accumulation of long-lived centrocytes because the BCL-2 protein inhibits programmed cell death (apoptosis), leading to longer cell life (Chap. 10).<sup>136</sup> The *BCL-2* rearrangement can be detected by the polymerase chain reaction or by iFISH testing (Chap. 10).

## BURKITT LYMPHOMA

In Burkitt lymphoma, the common genetic abnormality, is the translocation of the *MYC* oncogene from chromosome 8 to either the *Ig* heavy-chain region on chromosome 14, *t*(8;14)(q24;q32), or, less commonly,

the  $\kappa$  region on chromosome 2, *t*(2;8)(p13;q24), or the  $\lambda$  region on chromosome 22, *t*(8;22)(q24;q11). In the African endemic cases, the breakpoint on chromosome 14 includes the heavy-chain joining region, suggesting translocation occurs before complete *Ig* gene rearrangement in an early B cell (Chap. 13). In nonendemic cases, the translocation involves the *Ig* heavy-chain switch region, suggesting translocation occurs at a later stage of B-cell development.<sup>137</sup> EBV genomes are demonstrated in the tumor cells in most of the African cases, in approximately one-third of the cases associated with AIDS,<sup>138,139</sup> but less frequently in non-African, non-immune-deficient cases (Chap. 13).

## ANAPLASTIC LARGE CELL LYMPHOMA

The translocation *t*(2;5)(p23;q35) of anaplastic large cell lymphoma (ALCL) involves the nucleophosmin (*NPM*) gene at 5p35 and the ALCL tyrosine kinase (*ALK*) gene at 2p23,<sup>140</sup> leading to expression of the novel fusion protein p80, which can be identified by immunohistochemical studies.<sup>141</sup> This translocation has been identified in approximately 50 percent of systemic cases in adults and in a majority of pediatric cases of ALCL.<sup>142,143</sup> The *t*(2;5) translocation is rare in primary cutaneous ALCL, which is generally considered to be a different disease than systemic ALCL (Chaps. 14 and 15).<sup>144</sup>

## MARGINAL ZONE LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE

*t*(11;18)(*API2-MALT1*), *t*(1;14)(*IGH-BCL10*), *t*(14;18)(*IGH-MALT1*), and *t*(3;14)(*IGH-FOXP1*) occur in marginal zone B-cell lymphoma of MALT of different sites. The first three chromosome translocations are specifically associated with the marginal zone lymphoma of MALT



lymphomas, and the oncogenic products of these translocations target the nuclear factor- $\kappa$ B pathway (Chap. 12).<sup>101</sup>

## MANTLE CELL LYMPHOMA

t(11;14)(q13;q32) is present in the cells of most cases of mantle cell lymphoma and results in cyclin D1 upregulation. iFISH is the most useful test to identify the juxtaposition of the *CCND1* and *IGH* genes in mantle cell lymphoma (Chap. 11).<sup>145</sup>

## CLINICAL FEATURES

### HISTORY AND PHYSICAL EXAMINATION

A complete history and physical examination are required in all patients with lymphoma to ascertain the distribution of lymphadenopathy, extranodal disease, and functional disturbances of affected organ systems. It is also important to document whether the patient has fever (i.e., temperature  $>38^{\circ}\text{C}$  for 3 consecutive days), drenching night sweats, or metabolic wasting resulting in loss of more than 10 percent of body weight within the preceding 6 months. The presence of such “B symptoms” has unfavorable prognostic significance in HL, but recent analyses have shown that these B symptoms do not have independent prognostic significance for NHL, and current staging criteria no longer recommend assigning A or B designations when staging patients with NHL.<sup>17</sup> Fatigue, rash, pruritus, and alcohol-induced pain in patients with HL should also be noted, as their recurrence after treatment may herald disease relapse. During the physical examination, all enlarged lymph nodes ( $>1.5$  cm) should be recorded. Involved nodes typically are nontender, firm, and rubbery. The throat should be examined for involvement of the oropharyngeal lymphoid tissue (Waldeyer ring). Aggressive lymphomas more likely involve extranodal sites, such as the skin and CNS (see “Primary Extranodal Lymphoma”). Liver and spleen size should be assessed as well as palpation of the abdomen for evidence of enlargement of deep nodes (e.g., paraaortic, iliac).

### STAGING

Optimal management of a patient with lymphoma relies not only on knowledge of the precise histopathologic subtype of lymphoma, but also on an appreciation of the degree of disease dissemination, determined by a sequence of diagnostic tests known as “staging.” The 2014 recommendations from an international working group for the evaluation, staging, and response assessment of lymphomas emphasize the emerging dominance of 2-fluorodeoxyglucose (FDG)-PET/CT for initial staging and “end-of-treatment” response assessment of all FDG-avid lymphomas (including HL, DLBCL, follicular lymphoma, mantle cell lymphoma, Burkitt lymphoma, ALCL, and most subtypes of peripheral T-cell lymphoma) (Fig. 6–4).<sup>16,17</sup> Contrast-enhanced CT imaging remains the standard for lymphoma subtypes that are not reliably FDG avid (e.g., most marginal zone lymphomas, chronic lymphocytic leukemia/small lymphocytic lymphoma, lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, angioimmunoblastic T-cell lymphoma, mycosis fungoides, and cutaneous B-cell lymphomas). For FDG-avid lymphomas, PET/CT imaging has been shown to improve the accuracy of staging for both nodal and extranodal sites compared with CT imaging, leading to a change in stage in 10 to 30 percent of patients, usually as a result of upstaging. Alterations in the therapeutic plan occur in fewer patients, and no impact on overall survival as a result of PET/CT imaging has been demonstrated; however, improved staging ensures that fewer patients are undertreated or overtreated.<sup>17</sup> PET/CT is particularly important before consideration of radiation therapy for apparently localized disease because identification of disease

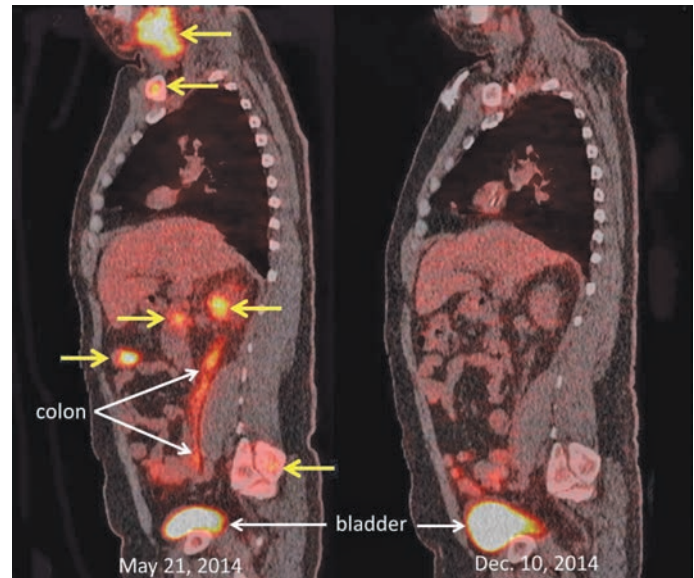


**Figure 6–4.**  $^{18}\text{F}$ fluorodeoxyglucose–positron emission tomography/computed tomography (FDG-PET/CT) imaging of Burkitt lymphoma before and after successful therapy. At the time of initial diagnosis (May 21, 2014), hypermetabolic areas of avid FDG uptake were detected in a right cervical mass, clavicle, abdominal lymph nodes, stomach, and pelvic bones (yellow arrows) in this patient with stage IV lymphoma. Physiologic FDG activity is also seen in the colon and bladder (white arrows). After completion of multiagent chemotherapy (December 10, 2014), there is no abnormal hypermetabolic activity in any of the original sites of disease, although physiologic FDG activity in the bowel and urinary system is still evident.

sites outside the radiation field by PET/CT entirely alters the treatment plan. The CT portion of a PET/CT scan may be performed with contrast enhancement at a full radiation dose to obtain a high-quality CT examination or without contrast using a lower radiation dose, which merely allows correction for the attenuation of radioactivity within the patient and to localize abnormalities seen on PET.

Full-dose, contrast-enhanced CT scans may identify additional findings, improve detection of abdominal or pelvic disease, permit radiation therapy planning in the treatment position, and are required for accurate nodal measurements on clinical trials. However, full-dose, contrast-enhanced CT scans entail additional radiation exposure and expense and uncommonly change the overall management plan. Several international consensus groups have recommended that PET/CT with full-dose, contrast-enhanced CT scans be done at the time of initial diagnosis, but that if contrast-enhanced CT scans do not divulge additional sites of disease, that only low-dose, noncontrast PET/CT imaging be done at the end of treatment.<sup>16</sup>

Although marrow aspiration and biopsy have been standard in lymphoma staging in the past, the high sensitivity of PET/CT for marrow involvement has rendered these procedures unnecessary for patients with HL and DLBCL who have negative PET/CT imaging of the bones and marrow. Data for other histologies are currently insufficient, and a single 2.5-cm core biopsy with flow cytometry and cytogenetics is still recommended for full staging of other subtypes.<sup>16,17</sup> Standard blood testing should also be performed, including a complete blood count (CBC) and chemistries. Lactate dehydrogenase and  $\beta_2$ -microglobulin are important serum prognostic markers that should be assessed at baseline for most lymphomas.<sup>33,146</sup> Table 6–3 lists the staging procedures that are

**TABLE 6-3.** Staging Procedures for Lymphoma

## Initial studies

History and physical examination  
 CBC  
 Metabolic panel, including renal and hepatic function  
 Uric acid  
 Lactate dehydrogenase and/or  $\beta_2$ -microglobulin  
 Hepatitis B and C serologies (if rituximab therapy planned)  
 HIV serology  
 Tumor biopsy specimen with histopathology  
 Flow cytometry of tumor specimen  
 Immunohistochemistry of tumor specimen  
 Cytogenetic analysis (including iFISH for lymphoma-associated translocations)  
 PET/CT scans of neck, chest, abdomen, and pelvis (for FDG-avid lymphomas)  
 Contrast-enhanced CT scans of neck, chest, abdomen, and pelvis (particularly for lymphomas that are not FDG avid)

## Additional studies (useful in selected cases)

Marrow aspiration and biopsy  
 Pregnancy testing in women of childbearing potential  
 Immunoglobulin and *TCR* gene rearrangement studies  
 Cardiac ejection fraction measurement (if anthracycline therapy planned)  
 Magnetic resonance imaging of brain if neurologic signs or symptoms  
 Cerebrospinal fluid analysis (including flow cytometry) for high-risk aggressive lymphomas or if neurologic signs or symptoms are present  
 Gastrointestinal studies (imaging and endoscopy) if Waldeyer ring involvement, mantle cell lymphoma, or enteropathy-associated lymphoma

CBC, complete blood count; CT, computed tomography; FDG, 2-fluorodeoxyglucose; iFISH, interphase fluorescence *in situ* hybridization; PET, positron emission tomography; TCR, T-cell receptor.

currently recommended, and the criteria used to assign a patient's stage are found in [Table 6-4](#).<sup>17</sup>

At the completion of therapy, all diagnostic studies performed at baseline detecting evidence of disease are repeated for response evaluation. Current recommendations suggest that PET/CT imaging be interpreted using visual inspection according to a 5-point "Deauville scale" ([Table 6-5](#)).<sup>15</sup> Deauville scores of 1 to 2 on FDG-PET scans indicate metabolic activity in tumor sites less than in the mediastinal blood pool, signifying complete metabolic response and complete remission. In contrast, Deauville scores of 4 or 5 at the end of treatment indicate residual abnormal metabolic activity, representing treatment failure ([Table 6-6](#)). A Deauville score of 3, indicating metabolic activity greater than the mediastinum but less than the liver, is indeterminant. Most patients with HL or DLBCL who have a Deauville score of 3 at the end of treatment have good outcomes, but careful followup of such patients is important.

The International Working Group (IWG) and the National Comprehensive Cancer Network (NCCN) have published recommendations for followup of patients in remission; the recommendations vary by histology, by whether a patient is on a clinical trial or not, and by

**TABLE 6-4.** The Lugano Staging System for Lymphomas<sup>17</sup>

| Stage*    | Involvement†                                                                       | Extranodal (E) Status                                                         |
|-----------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| LIMITED   |                                                                                    |                                                                               |
| I         | One nodal group involved                                                           | Single extranodal lesions without nodal involvement                           |
| II        | Two or more nodal groups involved, on the same side of the diaphragm               | Stage I or II nodal involvement with limited, contiguous extranodal extension |
| II bulky‡ | As in II above, but with "bulky" disease                                           | Not applicable                                                                |
| ADVANCED  |                                                                                    |                                                                               |
| III       | Involvement of nodal groups on both sides of the diaphragm§                        | Not applicable                                                                |
| IV        | Diffuse involvement of a visceral organ not contiguous with an involved nodal site | Not applicable                                                                |

CT, computed tomography; DLBCL, diffuse large B-cell lymphoma; FDG, 2-fluorodeoxyglucose; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; PET, positron emission tomography.

\*Stages are refined further for patients with HL by designating whether or not "B symptoms" are present, namely, fevers greater than 38.3°C, drenching night sweats, or unexplained weight loss of more than 10% of body mass over 6 months. Current recommendations discourage applying A and B designations to staging for patients with NHL because these features do not confer independent prognostic information.<sup>17</sup>

†Extent of disease is assessed by PET/CT imaging for FDG-avid lymphomas and by CT imaging for nonavid histologies.

‡A nodal mass of  $\geq 10$  cm, or greater than one-third of the transthoracic diameter at any level of thoracic vertebrae as determined by CT imaging, is considered bulky disease for HL. There is no consensus on the size of "bulk" for NHL, with a suggestion that 6 cm may be optimal for follicular lymphoma. Sizes between 6 and 10 cm have been advocated to define bulk for DLBCL.<sup>17</sup> Current recommendations are to record the longest measurement by CT scan and not employ the "X" notation to designate bulky disease. Stage II bulky disease may be considered to be either limited or advanced disease depending on histology and associated prognostic factors.

§Tonsils, Waldeyer ring, and spleen are considered nodal tissue in this staging system.

the clinical setting (e.g., initial vs. relapsed/refractory disease; complete response *or not*). For curable histologies such as HL and DLBCL, the likelihood of relapse decreases over time, and visits are reduced from every 3 months during the first 2 years, to every 6 months for the next 3 years, and then annually thereafter. Incurable histologies are observed every 3 to 6 months, determined by pretreatment risk factors, whether the patient is being managed conservatively, and whether treatment has achieved a complete remission or not. At each visit, a history, physical examination, CBC, metabolic panel, and serum lactate dehydrogenase are performed. The role, if any, of surveillance radiographic imaging for patients in remission is controversial. All groups strongly discourage surveillance monitoring with PET/CT imaging for patients in remission because of the high rate of false-positive findings in this setting, which

**TABLE 6-5.** The Deauville 5-Point Scale for Assessment of Positron Emission Tomographic/Computed Tomographic Imaging in Lymphoma Patients<sup>16</sup>

| Deauville Score | FDG Uptake*                                                                                                                       |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------|
| 1               | No significant FDG uptake in tumor site(s) above background                                                                       |
| 2               | FDG uptake in tumor site(s) less than that in the mediastinal blood pool                                                          |
| 3               | FDG uptake in tumor site(s) greater than in the mediastinum but less than in the liver                                            |
| 4               | FDG uptake in tumor site(s) moderately <sup>†</sup> higher than in the liver                                                      |
| 5               | FDG uptake in tumor site(s) markedly <sup>†</sup> higher than that in the liver and/or new FDG-avid lesions likely to be lymphoma |
| X               | New areas of uptake unlikely to be related to lymphoma                                                                            |

FDG, 2-fluorodeoxyglucose.

\*The Deauville 5-point scale scores the most intense uptake in a site of initial disease.

<sup>†</sup>It has been recommended that the Deauville score of 4 be applied to uptake in tumor site(s) that is less than twice as high as the maximum standard uptake value (SUV) in a large region of normal liver, whereas the score of 5 be used if the tumor uptake is more than twice the maximum SUV in the liver.

lead to unnecessary anxiety, expense, and biopsy procedures.<sup>15-17,33</sup> The Lugano IWG guidelines also discourage surveillance CT imaging for patients with HL and DLBCL in complete remission at the end of therapy. In contrast, NCCN guidelines recommend that contrast-enhanced CT imaging be performed no more frequently than every 6 months for 2 years after the end of therapy for DLBCL and HL and then be discontinued.

## ● PRIMARY EXTRANODAL LYMPHOMA

Lymphomas involving extranodal sites most commonly occur simultaneously with nodal involvement, either at the time of diagnosis or during the course of the disease. Extranodal involvement that occurs as the only initial evidence of lymphoma after staging procedures is referred to as *primary extranodal lymphoma*. The presence of a tumor or mass outside of the lymph nodes is usually not considered lymphoma until a biopsy is done and the histopathology establishes the diagnosis. On the other hand, solitary extranodal lymphomas can occur in virtually any organ or tissue and should be considered in the differential diagnosis of a solitary mass lesion anywhere. The histopathology of primary extranodal lymphoma is usually either marginal zone lymphoma of MALT or DLBCL. Follicular lymphoma and several other histologic subtypes of lymphoma may also occur. Therapy usually involves a combination of multidrug chemotherapy and a lymphocyte-directed monoclonal antibody, such as rituximab-cyclophosphamide, hydroxydoxorubicin, vincristine (Oncovin), and prednisone (R-CHOP). Selection of the best regimen depends on the histopathologic subtype of lymphoma and the location of the disease. Radiotherapy is used less commonly in the management of lymphoma than in the past because of concerns about induction of secondary malignancies and delayed cardiopulmonary toxicities, although it still has a role in treatment of localized,

stages I to II lymphomas and for consolidation of bulky adenopathy (>10 cm) in selected settings.

An unanswered pathogenetic question concerning primary extranodal lymphoma is the propensity for both sites of paired organs (e.g., ovaries, testicles, breasts, ocular adnexa, adrenal glands, kidneys, and ureters) to be affected simultaneously. It is also curious that several of these sites (e.g., kidney) are normally devoid of significant accumulations of lymphatic tissue. If the transformed lymphocyte arises outside these tissues, it must have a tropism for both paired organs, perhaps because of expression of site-specific adhesion molecules or addressins.<sup>147</sup>

## CENTRAL NERVOUS SYSTEM

Primary lymphomas originating in and confined to the leptomeninges,<sup>148</sup> brain,<sup>149-151</sup> or spinal cord<sup>152</sup> are uncommon. They almost always are of an aggressive histologic subtype, usually DLBCL.<sup>151,153</sup> Spinal cord compression typically presents with back pain, followed by extremity weakness, paresis, and paralysis. Leptomeningeal spread may present with cranial nerve palsies and signs of meningeal irritation, for example, headache and stiff neck. Intracerebral mass lesions may present with headaches, lethargy, papilledema, focal neurologic signs, or seizures. Intracerebral lymphoma increased dramatically after the onset of the HIV epidemic as a result of the association with AIDS-related aggressive lymphomas. The incidence of intracerebral lymphoma has slowed in AIDS patients because of more successful antiviral therapy. Primary pituitary (or hypothalamic) extranodal lymphoma may result in hypopituitarism. Diabetes insipidus or anterior pituitary failure may occur. The lesion may invade the sella turcica or other neighboring bone and nervous tissue.<sup>154-156</sup>

## EYE

Ophthalmic lymphoma, the most common orbital malignancy, includes lymphoma localized to the eyelid, conjunctiva, lacrimal sac, lacrimal gland, orbit, or intraocular space.<sup>157-159</sup> This location accounts for approximately 7 percent of all extranodal lymphomas.<sup>160</sup> The most frequent subtype is extranodal marginal zone lymphoma of MALT. Bilateral involvement occurs in 10 percent of cases. The most common site of ocular lesions is the periorbital soft tissues, particularly the conjunctival mucosal surfaces and the area surrounding the lacrimal gland. These lesions typically have a low risk of progression and commonly have the histology of a marginal zone lymphoma of MALT or follicular center cell lymphoma and may be associated with *C. psittaci* (see "Infectious Agents" section). In a Danish study, approximately 50 percent of orbital and ocular adnexal lymphomas were of the marginal zone lymphoma of MALT subtype; DLBCL was the most common intraocularly.<sup>91,160</sup> Lymphoma arising in the lacrimal sac was usually DLBCL. There has been a striking increase in incidence rates for lymphoma of the eye over the past 30 years.<sup>24,157-159,161</sup> Patients with marginal zone lymphoma of MALT of the ocular orbit may relapse or have progression of disease after initial therapy, and relapses can be found at extraocular sites.<sup>91</sup> Overall survival, however, was not significantly worse for patients with relapse. The frequency of translocations involving the *MALT1* and *IGH* gene loci is low in orbital marginal zone lymphoma of MALT (approximately 5 percent) but may predict increased risk of relapse.<sup>160</sup> The therapy for orbital marginal zone lymphoma is usually radiotherapy, which is curative in the majority of patients.<sup>157</sup> Anecdotal reports of responses to rituximab or rituximab postradiation suggest that rituximab may have a therapeutic role in low-grade lymphomas involving the eye. In the rare situation where DLBCL involves the periorbital soft tissue, treatment is determined by the distribution of the disease, but R-CHOP chemotherapy either alone or combined with local radiotherapy is standard.



**TABLE 6–6.** Revised Criteria for Lymphoma Response Assessment<sup>17</sup>

| Response (By Site)                   | PET/CT-Based Response                                                                                                                 | CT-Based Response                                                                                                                                                                                                                           |
|--------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Complete Remission</b>            | Complete metabolic response                                                                                                           | Complete radiologic response                                                                                                                                                                                                                |
| Lymph nodes and extranodal (E) sites | Deauville score of 1, 2, or 3 with or without a residual, imaged mass                                                                 | Target nodes regress to $\leq 1.5$ cm in longest diameter<br>No extranodal sites                                                                                                                                                            |
| Nonmeasured lesion                   | Not applicable                                                                                                                        | Absent                                                                                                                                                                                                                                      |
| Organ enlargement                    | Not applicable                                                                                                                        | Regress to normal                                                                                                                                                                                                                           |
| New lesions                          | None                                                                                                                                  | None                                                                                                                                                                                                                                        |
| Marrow                               | FDG negative                                                                                                                          | Normal morphology                                                                                                                                                                                                                           |
| <b>Partial Remission</b>             | Partial metabolic response                                                                                                            | Partial radiologic response                                                                                                                                                                                                                 |
| Lymph nodes and extranodal (E) sites | Deauville score of 4 or 5 with reduced uptake compared to baseline                                                                    | $\geq 50\%$ decrease in the sums of the biperpendicular diameters (SPD) of up to 6 target measurable lesions                                                                                                                                |
| Nonmeasured lesion                   | Not applicable                                                                                                                        | Absent, normal, or regressed without increase                                                                                                                                                                                               |
| Organ enlargement                    | Not applicable                                                                                                                        | Spleen has regressed by $\geq 50\%$ in length beyond normal                                                                                                                                                                                 |
| New lesions                          | None                                                                                                                                  | None                                                                                                                                                                                                                                        |
| Marrow                               | Reduced FDG uptake compared to baseline, but higher than in normal marrow                                                             | Not applicable                                                                                                                                                                                                                              |
| <b>No Response or Stable Disease</b> | No metabolic response                                                                                                                 | Stable disease                                                                                                                                                                                                                              |
| Lymph nodes and extranodal (E) sites | Score 4 or 5 without significant change in FDG uptake compared to baseline                                                            | $< 50\%$ decrease in the sums of the biperpendicular diameters (SPD) of up to 6 target measurable lesions                                                                                                                                   |
| Nonmeasured lesion                   | Not applicable                                                                                                                        | No increase consistent with progression                                                                                                                                                                                                     |
| Organ enlargement                    | Not applicable                                                                                                                        | No increase consistent with progression                                                                                                                                                                                                     |
| New lesions                          | None                                                                                                                                  | None                                                                                                                                                                                                                                        |
| Marrow                               | No change from baseline                                                                                                               | Not applicable                                                                                                                                                                                                                              |
| <b>Progressive Disease</b>           | Progressive metabolic disease                                                                                                         | Progressive disease                                                                                                                                                                                                                         |
| Lymph nodes and extranodal (E) sites | Score 4 or 5 with significant increase in FDG uptake compared to baseline and/or new FDG-avid foci consistent with new lymphoma sites | Target lesions with an increase of $> 50\%$ from nadir with a longest diameter of at least 1.5 cm. Increases must be by at least 0.5 cm for lesions $< 2.0$ cm and by at least 1.0 cm for lesions $> 2.0$ cm. New or recurrent splenomegaly |
| Nonmeasured lesion                   | None                                                                                                                                  | New or clear progression of preexisting nonmeasured lesions                                                                                                                                                                                 |
| New lesions                          | New FDG-avid foci consistent with lymphoma and not suggestive of other etiologies (infection, inflammation)                           | A new lesion $> 1.0$ cm (or if $< 1.0$ , must be demonstrated to be due to lymphoma by biopsy or other unequivocal method)                                                                                                                  |
| Marrow                               | New or recurrent FDG-avid foci                                                                                                        | New or recurrent involvement                                                                                                                                                                                                                |

FDG, 2-fluorodeoxyglucose.

Intraocular lymphomas are a rare presentation of lymphoma of the eye. Most cases are DLBCL. The diagnosis is established by a vitrectomy. There is an approximately 50 percent chance that the disease will be bilateral, and the disease is frequently associated with brain or leptomeningeal involvement. The mainstay of therapy in the past has been local radiotherapy or intraocular injections of methotrexate or rituximab, but most patients treated in this manner relapse within the eye or brain. Standard chemotherapeutic agents administered intravenously (e.g., R-CHOP) typically do not penetrate the eye or brain, rendering these regimens ineffective. Many neuro-oncologists have advised treating intraocular lymphomas similarly to primary DLBCL of the brain with high-dose methotrexate-based chemotherapy with or without intrathecal treatment and/or whole-brain and eye radiotherapy. A large study conducted at 17 European centers did not confirm the expected efficacy of this aggressive approach for vitreoretinal lymphoma, however.<sup>158</sup>

## PARANASAL SINUSES

Localized NHL involving the nasal cavity and/or paranasal sinuses may be DLBCL, T-cell lymphoma, or NK/T-cell lymphoma.<sup>74,162–168</sup> The nasal cavity is the predominant site of involvement in T-cell and NK/T-cell lymphoma, whereas sinus involvement without nasal disease is common in B-cell lymphoma. Systemic B symptoms are more frequently observed in NK/T-cell lymphoma. Based on *in situ* hybridization studies, there is a strong association of EBV with NK/T-cell lymphoma. These lymphomas may involve the frontal, maxillary, ethmoid, and sphenoid sinuses and typically involve bone. They present with local pain, upper airway obstruction, rhinorrhea, facial swelling, or epistaxis. They may extend into the periorbital area, causing proptosis, visual loss, or diplopia. These lymphomas typically are DLBCL in the United States and Western Europe and more often T- and NK-cell lymphomas in Asia.

Nasal NK/T lymphomas are typically treated with combination chemotherapy, including L-asparaginase plus radiotherapy.<sup>162,169</sup> In contrast, patients with primary sinus lymphoma usually have DLBCL. A series of 80 patients with primary sinonasal DLBCL treated with R-CHOP chemotherapy demonstrated a long-term progression-free survival rate of 50 to 60 percent, with only a single patient experiencing CNS relapse.<sup>170</sup>

## SKIN

The three main types of cutaneous B-cell lymphomas are primary cutaneous marginal zone B-cell lymphoma, primary cutaneous follicular center lymphoma, and primary cutaneous large B-cell lymphoma (leg type) as defined by the WHO–European Organization for Research and Treatment of Cancer.<sup>171</sup> Primary cutaneous marginal zone B-cell and primary cutaneous follicle center lymphoma are indolent types with an excellent prognosis that should be treated primarily with nonaggressive therapies, including simple excision, glucocorticoid injections, or local radiotherapy. Primary cutaneous large B-cell lymphoma (leg type) is a diffuse dermal infiltrate of neoplastic B cells with extension to both the papillary dermis and the subcutaneous fat.<sup>172,173</sup> It typically presents as a solitary soft-tissue mass and mimics a soft-tissue sarcoma until biopsy clarifies the diagnosis. It is an aggressive lymphoma that should be treated primarily with aggressive chemotherapy (Chap. 9). Chapter 14 discusses classical T-cell cutaneous lymphomas, particularly mycosis fungoides.

## CHEST AND LUNG

Primary pulmonary lymphoma may present as a pulmonary nodule or mass and may be associated with hilar lymph node enlargement. The histopathology is usually marginal zone B-cell lymphoma of the MALT or DLBCL, though lymphomatoid granulomatosis may also present in this fashion.<sup>174,175</sup> Lung biopsy is usually required to make a definitive diagnosis. Pleural effusions may occur as a result of either central lymphatic obstruction or pleural seeding.

Primary chest wall lymphoma may present as local pain or may be accompanied by fever, sweating, and dyspnea. These masses usually require excisional or incisional biopsy. They may be associated with pleural effusions and involvement of neighboring ribs.<sup>176</sup>

Primary endobronchial lymphoma is a rare occurrence and may follow lung transplantation. It can lead to airway obstruction as an early sign.

## HEART

Primary cardiac lymphoma may involve the heart, pericardium, or both. Patients may present with dyspnea, edema, arrhythmia, or pericardial effusion. The effusion may result in cardiac tamponade. Lymphomatous masses may be found in the right atrium (most common), pericardium, right ventricle, left atrium, or left ventricle. Most cases are a B-cell lymphoma; less than 5 percent are of T-cell lineage.<sup>177–179</sup>

## GASTROINTESTINAL TRACT

Gastrointestinal lymphoma is the most common form of extranodal lymphoma, accounting for one-third of cases.<sup>180</sup> The most commonly involved site is the stomach, followed by the small bowel, ileum, cecum, colon, and rectum. Lymphoma of the stomach typically causes dyspeptic symptoms and sometimes anorexia or early satiety. Hemorrhage is unusual but if present suggests a high-grade lymphoma. Diagnosis typically is made by endoscopic biopsy.<sup>180,181</sup> *H. pylori* infection has been implicated in the pathogenesis of MALT gastric lymphoma.<sup>88</sup> At endoscopy, mild-to-severe gastritis is common. Multiple biopsies are

important for obtaining adequate material to determine the presence of *H. pylori*. MALT lymphoma is common, but DLBCL also may arise *de novo* or may be found in the background of a MALT lymphoma.<sup>181</sup> If both subtypes of lymphoma are present, the treatment should be directed at the large B-cell lymphoma.

In the bowel, the small intestine, rectum, and colon may be involved, in that order of frequency.<sup>88,182</sup> The intestinal location most often involved is the ileocecal region, followed by small bowel, large bowel, and multiple intestinal sites.<sup>182</sup> Primary esophageal lymphoma is rare.<sup>183</sup> Primary colonic lymphoma is associated with symptoms of diarrhea, lower gastrointestinal bleeding, and nausea and vomiting secondary to low-grade obstruction. The most common disease location is the cecum, followed by the right colon and the sigmoid colon.<sup>184</sup>

Rare cases of lymphoma may be confined to the liver. Right upper quadrant pain is the most common symptom. In about half of cases, there is a history of previous inflammatory liver disease, such as hepatitis C.<sup>185–188</sup>

Primary extranodal lymphoma of the pancreas may present with abdominal pain, nausea, vomiting, obstructive jaundice, and weight loss and very rarely signs of pancreatitis.<sup>189–191</sup>

The gallbladder may be the site of primary extranodal lymphoma and may present with right upper quadrant pain or other symptoms and signs consistent with cholecystitis.<sup>192,193</sup> It may extend into the bile ducts with jaundice and other signs of bile duct obstruction.<sup>194</sup>

## GENITOURINARY

### Testicular

Primary lymphoma of the testes typically presents as a painless enlargement of the testis in an older man (Fig. 6–5). A hydrocele may also be present.<sup>195–198</sup> The histologic type usually is a DLBCL. At presentation, two-thirds of cases are localized to the testicle or to the testicle and pelvic or abdominal lymph nodes. After orchiectomy has established the diagnosis, patients are staged with a special focus on the remaining testicle. If sonography of the remaining testicle demonstrates a solid mass, it should be assumed to be lymphoma. Patients presenting with testicular lymphoma have historically experienced a poorer prognosis compared to patients with other presentations of DLBCL, with relapses commonly occurring in either the CNS or contralateral testicle.<sup>199</sup> The current international standard of care consists of orchiectomy, R-CHOP chemotherapy given every 21 days for six cycles, intrathecal methotrexate, and locoregional radiotherapy to the contralateral testicle.<sup>195,196</sup> This combined modality approach has been shown to render an excellent 5-year progression-free survival rate of 74 percent.<sup>196</sup>

### Ovary

Primary lymphoma of the ovary is often bilateral and presents as an abdominal mass with abdominal pain or palpation of a mass on physical examination.<sup>200–205</sup>

### Uterus, Cervix, Genitalia

Cases of lymphoma limited to the uterus,<sup>206–208</sup> uterine cervix,<sup>209,210</sup> vagina, or vulva<sup>211</sup> can occur. Uterine and cervical lymphoma usually present with an abdominal mass or vaginal bleeding. Lymphoma can develop within a uterine leiomyoma.<sup>208</sup>

### Kidney

Lymphomatous involvement of both kidneys usually presents with renal insufficiency, which can be reversed with multidrug chemotherapy or radiotherapy. Bilateral enlargement of the kidneys without obstruction and other organ or nodal involvement and absence of other causes of renal failure are characteristic of primary renal lymphoma.<sup>212–216</sup>



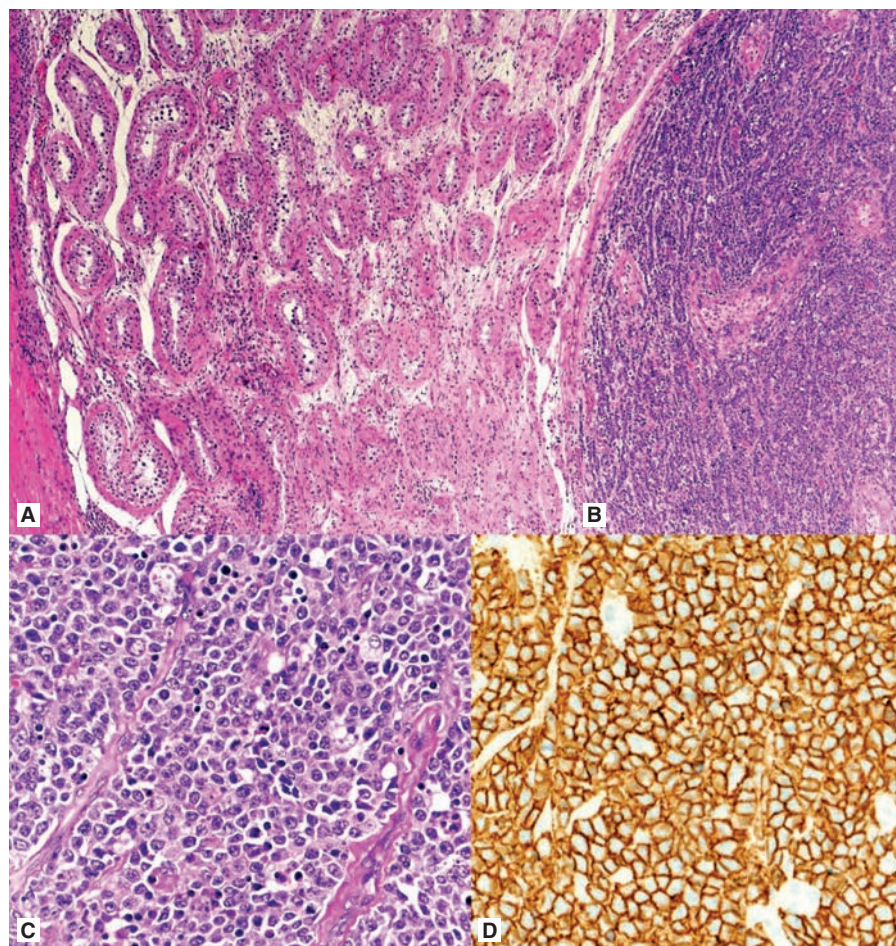


**Figure 6-5.** Primary extranodal diffuse large B-cell lymphoma in a testicular mass. **A.** Uninvolved area of testes. **B.** Area of testes replaced by an abnormal lymphocyte population. **C.** High-power view of the lymphoma cells. **D.** Positive immunohistochemistry staining for CD20. (Used with permission from Raymond Felgar, University of Pittsburgh Medical Center.)

The origins of the lymphoma are perplexing as the kidney is thought to be devoid of lymphoid tissue. Both careful staging and postmortem examination have verified the absence of lymphoma in other sites. An increased association of renal cell carcinoma and primary renal lymphoma may exist.<sup>215</sup> Rarely, the lymphomatous involvement, although still solely extranodal, involves only the perirenal space.<sup>217</sup>

#### **Ureter, Bladder, Prostate**

Bilateral ureteral involvement with obstructive renal failure may occur.<sup>218</sup> Primary lymphoma of the bladder may rarely extend to the kidney. Usually, it is localized and responds well to treatment.<sup>219–221</sup> Primary extranodal lymphoma may involve the prostate.<sup>222,223</sup>

#### **SPLEEN**

Primary splenic lymphoma is rare.<sup>224,225</sup> Concomitant marrow involvement is present in most cases. When primary to the spleen, the lymphoma may be principally confined to the red pulp and is usually consistent histopathologically with DLBCL or marginal zone lymphoma.<sup>224</sup> In the absence of lymph node involvement or splenic white pulp involvement at the time of diagnosis or during the course of the disease, it can be considered an “extranodal” splenic lymphoma.<sup>225</sup>

#### **BONE**

Primary bone lymphoma may involve any bone but usually affects the long bones.<sup>226–229</sup> The presentation is usually bone pain, and the lesions are usually lytic when imaged.<sup>226,228</sup> When the skull is involved, the lymphoma may invade the CNS.<sup>227</sup> Skeletal involvement exhibited in most patients is due to aggressive lymphomas, particularly DLBCL. Management generally involves R-CHOP chemotherapy with consolidative radiotherapy.<sup>226</sup>

#### **BREAST**

The clinical presentation of primary lymphoma of the female breast often mimics carcinoma of the breast.<sup>229a</sup> A small proportion of cases may be bilateral. The pathologic diagnosis is DLBCL in approximately 85 percent of cases.<sup>230</sup> BCL-2 expression is frequently present in the tumor cells. Small lymphocytic lymphoma, follicular lymphoma, and marginal zone lymphoma of MALT may also be the histopathologic diagnosis.<sup>231,232</sup> Staging may uncover nodal involvement, marrow involvement, or other extranodal sites with lymphoma in as many as half of cases. Approximately 10 percent of cases of primary breast lymphoma relapse in the CNS.<sup>230,232</sup> An uncommon type of T-cell anaplastic lymphoma of the breast may be the consequence of certain types of breast reconstructive implants.<sup>232a–232c</sup>



## ENDOCRINE GLANDS

Primary adrenal lymphoma usually present bilaterally and thus may lead to adrenal insufficiency. In the latter case, the presenting symptoms may be fatigue, asthenia, and other signs of hypoadrenocorticism.<sup>233–236</sup> Primary thyroid lymphoma often occurs in a gland afflicted by Hashimoto thyroiditis. Thus, it occurs more frequently in women than men. The patient may present with an enlarged thyroid (goiter) or have symptoms as a result of tracheal compression.<sup>237–239</sup> The histopathology may be DLBCL or marginal zone B-cell MALT lymphoma. Primary pituitary lymphoma is discussed under “Central Nervous System.”

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## CHAPTER 7

# PATHOLOGY OF LYMPHOMAS

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### SUMMARY

The World Health Organization (WHO) classification of lymphoid neoplasms has gained worldwide acceptance by both pathologists and oncologists. As further advances in the classification of lymphoma are agreed upon, the WHO provides an updated version of its classification. The most recent modification was published in 2016. It provides a list of distinct lymphocytic neoplasms that are defined by a combination of morphologic, immunophenotypic, genetic, and clinical features and correlates each disease with a cell of origin in the lymphopoietic hierarchy. Because the classification of lymphomas requires the integration of such diverse information, the diagnosis of specific lymphomas has become more complex compared to other malignancies. As a result, several ancillary studies have become essential in the diagnosis of a specific pathological type of lymphoma, requiring special handling of biopsy material when a diagnosis of lymphoma is suspected. The WHO classification identifies three major categories of lymphoid malignancies: B-cell neoplasms, T- and natural-killer (NK) cell neoplasms, and Hodgkin lymphoma. Two major categories are identified within the B-cell and T-/NK-cell neoplasms: precursor neoplasms and peripheral or mature neoplasms. Unlike previous lymphoma classifications, the WHO classification does not group different lymphomas by clinical outcome or histologic grade. It recognizes that each disease has distinctive clinical features and response to treatment and may have a spectrum of rate of clinical progression that may correlate with histologic grade or gene expression patterns. New approaches for classifying lymphoma include the study of gene expression profiling by complementary DNA microarray technology, which provides additional insights into the subclassification of diseases such as diffuse large B-cell lymphoma and chronic lymphocytic leukemia. Proteomic approaches will add further texture to the molecular taxonomy of lymphoma classification.

**Acronyms and Abbreviations:** ABC, activated B cell; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; FISH, fluorescence *in situ* hybridization; GCB, germinal center B cell; IGH, immunoglobulin heavy chain; LP, lymphocyte predominant; MALT, mucosa-associated lymphoid tissue; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NK, natural killer; PCR, polymerase chain reaction; PTCL, peripheral T-cell lymphoma; REAL, Revised European-American Lymphoma; WHO, World Health Organization; ZAP-70, zeta-associated protein of 70 kDa.

## HISTORICAL ASPECTS OF LYMPHOMA CLASSIFICATION

The classification of malignant lymphoma had been fraught with controversy during much of the 20th century, but consensus has been reached during the past two decades. A detailed discussion of the history of lymphoma classification is beyond the scope of this chapter and can be found elsewhere.<sup>1,1a</sup>

From Thomas Hodgkin's description in 1832 of what became known as Hodgkin disease<sup>2</sup> to the first half of the 20th century, several types of lymphomas with distinctive morphologic and clinical features were described using a variety of terms, including lymphoma, lymphosarcoma, reticulum cell sarcoma, and giant follicular lymphoma.<sup>1,1a</sup> However, many of the terms were not used uniformly, resulting in significant misunderstanding, particularly between pathologists and clinicians. Starting in the 1930s, several attempts were made to classify lymphomas and provide some uniformity of diagnosis, culminating in the Rappaport classification, initially published in 1956, which divided lymphomas based on growth pattern, cell type, and stage of differentiation.<sup>3,4</sup> Most important, this classification demonstrated clinical relevance, showing that lymphomas with a nodular pattern had a better prognosis than diffuse lymphomas.

In the 1960s and 1970s, advances in our understanding of the immune system and its cellular composition had a profound effect on our understanding of lymphocyte biology and a consequent effect on our understanding of malignant lymphomas. Normal lymphocytes could now be classified into distinct lineages (B, T, and natural killer [NK]), which could be determined by expression of lineage-specific surface antigens and eventually by genetic analysis of B- and T-cell receptors.<sup>5,6</sup> Several new lymphoma classification schemes were developed to incorporate the new immunologic data, the most important being the Kiel classification<sup>7</sup> (used primarily in Europe) and the Lukes and Collins classification<sup>8</sup> (used primarily in North America). By the 1970s, at least five classification schemes were widely used in different parts of the world. At the same time, clinical studies were beginning to show that some patients with aggressive lymphomas could be cured with combination chemotherapy.<sup>9</sup> Oncologists needed to interpret results of clinical trials performed in different institutions, a situation made difficult by the use of different classification schemes that were not easily translated among themselves.

The problem was addressed by the United States National Cancer Institute, which convened a large group of investigators to determine which classification scheme was best at predicting clinical outcome of lymphoma. None of the classification schemes was identified as predicting clinical outcome better than the other schemes. Therefore, pathologists were advised to continue using one of the six classification schemes studied, and the "Working Formulation" was developed so that oncologists could translate clinical data derived in different institutions using different classification schemes.<sup>10</sup> Lymphomas were divided into 10 categories based solely on morphologic features. To help clinicians deal with a large number of lymphoma subtypes, the lymphomas were further grouped into three clinical prognostic groups (clinical grades). Although the Working Formulation was not intended to be a stand-alone classification scheme, it was used as such by many institutions, particularly in North America.

However, increasing phenotypic and genotypic data were further defining several distinctive lymphoma subtypes. The Working Formulation lumped different lymphomas into broad categories that were obscuring the distinctive features of the newly described entities. The Working Formulation categories were based solely on morphologic features and were not able to incorporate new immunologic and molecular genetic data that were recognizing new types of lymphomas.

In the 1980s and 1990s, several new lymphoma entities were identified based on new immunologic and molecular genetic data. Although attempts were made to incorporate these new entities into the existing classification schemes,<sup>11</sup> problems with uniformity between different institutions persisted. A desire to eliminate the continued confusion ultimately led to a new approach to lymphoma classification proposed by the International Lymphoma Study Group that used all available information, including morphology, immunophenotype, genetic, and clinical features, to define a list of distinctive entities that could be uniformly diagnosed by hematopathologists. The proposal was published in 1994 and was known as the Revised European-American Lymphoma (REAL) classification.<sup>12</sup> Importantly, this classification identified entities that had distinctive clinical features and could be reproducibly diagnosed by expert hematopathologists.<sup>13</sup>

## ● WORLD HEALTH ORGANIZATION CLASSIFICATION

In the late 1990s, a new World Health Organization (WHO) classification for lymphoproliferative disorders was being developed, based on the REAL classification. First published in 2001 (and revised in 2008<sup>14</sup> and 2016<sup>14a</sup>), the WHO classification represented a consensus between an international group of more than 50 experienced hematopathologists, including contributions from a clinical advisory committee of hematologists and oncologists experienced in treating lymphomas. The WHO classification (Chap. 1) identified several major categories, including precursor lymphoid neoplasms, mature B-cell neoplasms, mature T- and NK-cell neoplasms, and Hodgkin lymphoma.<sup>14a</sup>

Distinctive lymphoma entities were identified based upon a combination of morphologic, immunophenotypic, genetic, and clinical features. The 2008 WHO classification included several provisional entities and categories of unclassifiable neoplasms with features intermediate between two distinct entities. This allows the classification to retain flexibility so that new data that further identify distinct diseases within these entities can be incorporated, as was done in the 2016 revision.<sup>14a</sup> In distinction from the Working Formulation, lymphomas were not classified based on clinical outcome. The WHO classification agreed that each type of lymphoma that was identified by pathologic and clinical features

could have a spectrum of clinical aggressiveness, and that lumping distinct entities into groups based on clinical outcome would inhibit the development of targeted therapeutic approaches. Therefore, the WHO classification represents a complete change from the Working Formulation, with the emphasis on pathologic classification rather than classification based on survival characteristics.

Genome-wide expression studies have been instrumental in further delineating distinctive subtypes of lymphomas of clinical relevance. Using complementary DNA microarray technology, the expression of thousands of genes at the mRNA level can be studied simultaneously and compared to other tumor samples.<sup>15</sup> Such studies have (1) defined more than one distinct entity in what was previously characterized as a morphologically homogeneous category, (2) identified distinct gene expression patterns that each encompasses a disease that may demonstrate morphologic heterogeneity, and (3) identified new surface molecules and signaling pathways that could provide targets for new therapeutic approaches. The impact of the new gene expression profiles is detailed in the sections on separate lymphomas in this chapter. Table 7-1 gives a comprehensive list of pathological lymphoma types and the immunophenotypic, cytogenetic, and genetic abnormalities closely associated with the morphological type. Each chapter discussing specific lymphoma types also gives comprehensive information on correlative immunophenotypic, cytogenetic, and genetic abnormalities associated with each type of lymphoma.

The WHO classification attempts to correlate each lymphoma to normal lymphocyte biology by postulating a cell of origin for each neoplasm. This correlation is particularly well suited for B-cell lymphomas in which several distinct stages of normal B-cell development can be identified (Fig. 7-1) but is not as satisfying for T and NK neoplasms. Briefly, B-cell development begins in the marrow with precursor B lymphoblasts that differentiate into naïve B cells that circulate in the blood. The lymph node is the primary site where B cells encounter antigen, where naïve B cells colonize primary follicles and secondary follicles in mantle zones (Figs. 7-2 to 7-4). Upon antigen stimulation, these cells undergo blast transformation and enter the germinal center reaction in the late primary immune response and the secondary immune response. In the germinal center, cells downregulate BCL2 (Fig. 7-5) and initially transform into intermediate-size cells (follicular B blasts), then into large centroblasts, and finally into small centrocytes (Figs. 7-6 and 7-7).<sup>16</sup> Cells that survive the germinal center upregulate BCL2 and

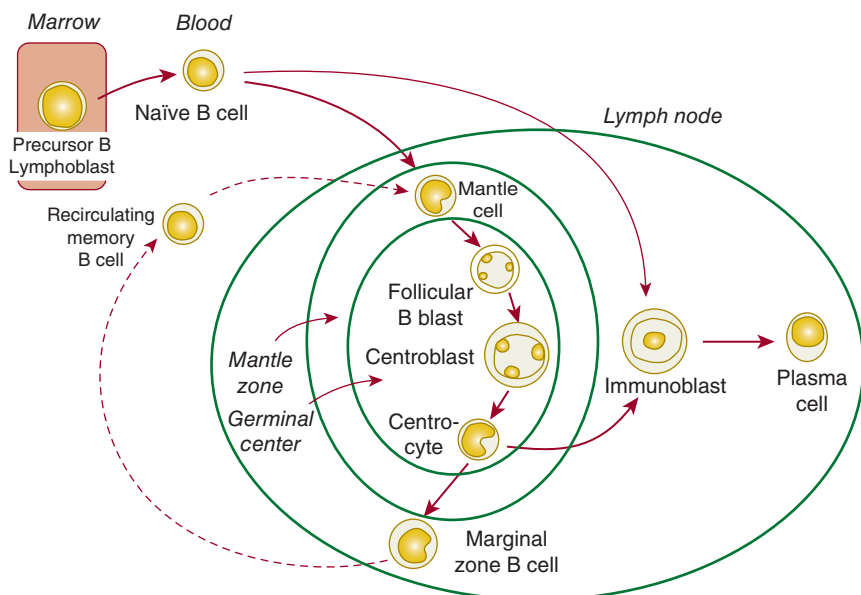
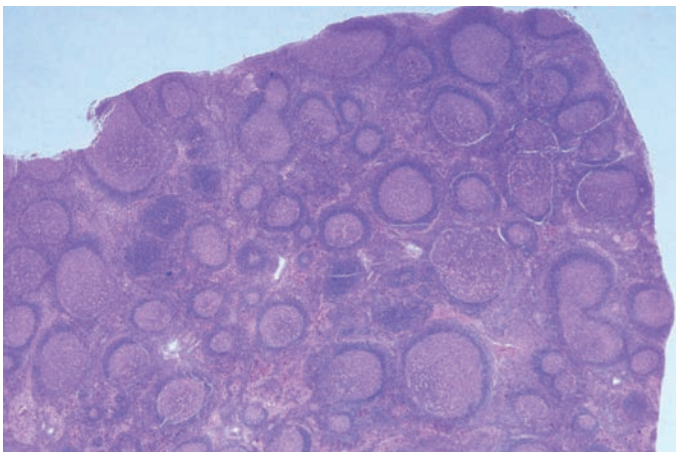
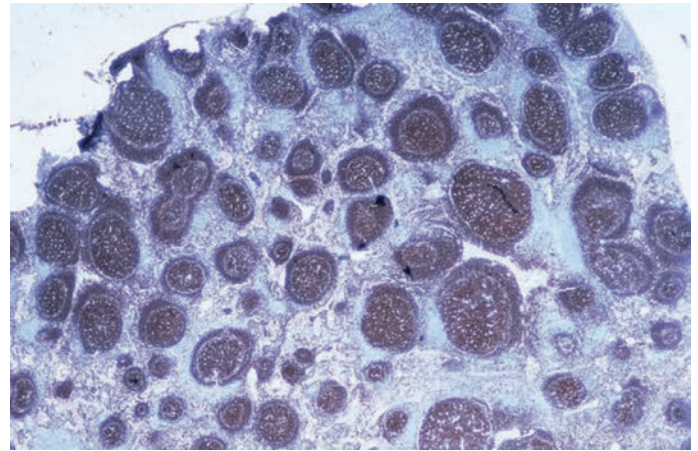
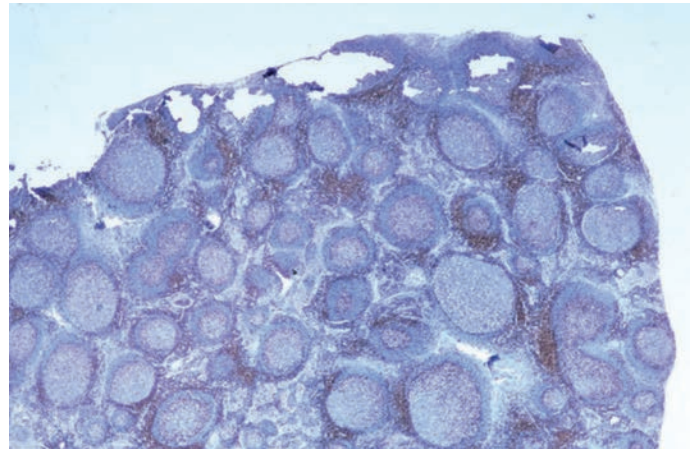
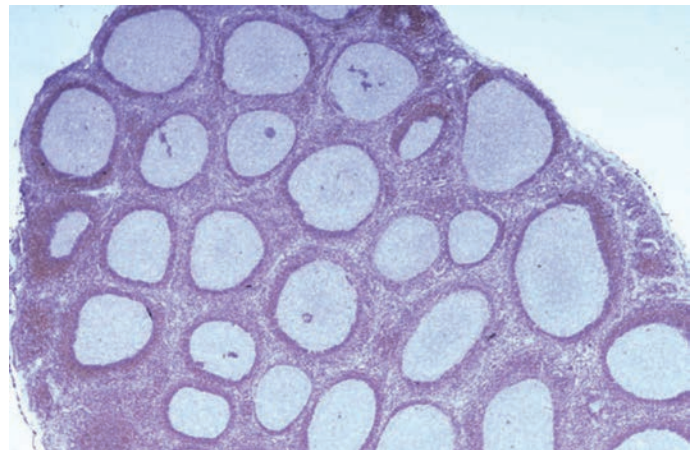


**Figure 7-1.** Stages of B-cell development. Precursor B lymphoblasts in the marrow differentiate into mature B cells that circulate in blood and colonize mantle zones of lymphoid follicles. Upon antigen stimulation, the cells can differentiate directly into immunoblasts (early primary immune response) or enter the germinal center reaction (late primary and secondary immune responses). In the germinal center, cells undergo blast transformation and progress to form large centroblasts, followed by small centrocytes. These cells differentiate into either antibody-secreting plasma cells through an immunoblast stage or memory B cells that can recirculate or localize to the marginal zones of lymphoid follicles.



**TABLE 7-1.** Standard and Ancillary Studies for Lymphoma Diagnosis

| Method                                   | Applications                                                                                                                                                              | Type of Tissue Needed                                                                                |
|------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------|
| Routine histology                        | Examination of routine sections will allow diagnosis of lymphoma in certain situations. In the remaining cases, the diagnosis will require the use of ancillary studies   | Formalin fixed                                                                                       |
| Immunohistochemistry                     | Immunophenotyping for lymphoma classification; can demonstrate B-cell clonality (light-chain restriction) and unique antigen expression in some cases                     | Formalin fixed                                                                                       |
| Automated flow cytometry                 | Demonstration of B-cell clonality by surface immunoglobulin (Ig) light-chain restriction; immunophenotyping for lymphoma classification                                   | Fresh tissue (single-cell suspensions)                                                               |
| Polymerase chain reaction analysis       | Demonstration of B- and T-cell clonality by Ig and T-cell receptor analyses; demonstration of lymphoma-specific translocations (example: <i>BCL2</i> gene rearrangements) | Frozen tissue<br>Can be performed on paraffin tissue but may not yield amplifiable DNA in some cases |
| Cytogenetics                             | Demonstration of clonality; demonstration of lymphoma-specific translocations                                                                                             | Sterile fresh tissue                                                                                 |
| Fluorescent <i>in situ</i> hybridization | Demonstration of lymphoma-specific translocations                                                                                                                         | Fresh tissue<br>Can be performed on paraffin tissue, but yield is variable                           |

**Figure 7-2.** Reactive lymph node with follicular hyperplasia, characterized by numerous secondary lymphoid follicles with intact mantle zones.**Figure 7-3.** Same reactive lymph node as in Fig. 7-2 but stained with an antibody to CD20 (B-cell marker), showing B cells predominantly localized to the follicles.**Figure 7-4.** Same reactive lymph node as in Fig. 7-2 but stained with an antibody to CD3 (T-cell marker), showing T cells predominantly localized to the interfollicular areas.**Figure 7-5.** Same reactive lymph node as in Fig. 7-2 but stained with an antibody to the antiapoptotic protein BCL2. Note the negative staining of the germinal centers where most of the cells will die during the maturation process.



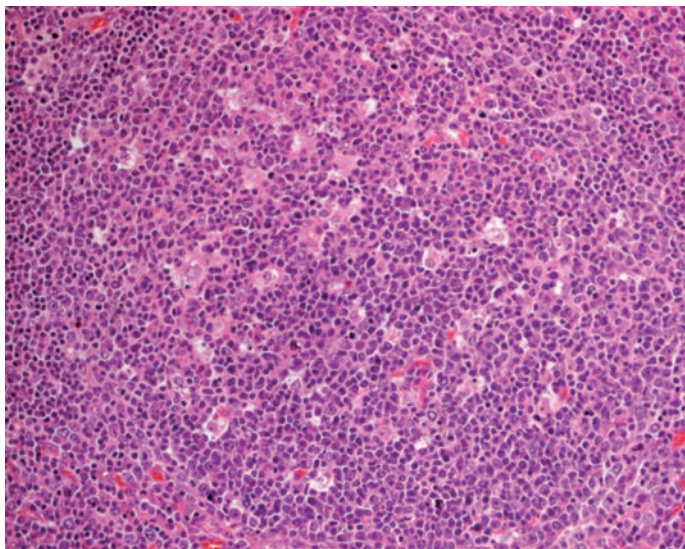


**Figure 7-6.** Reactive germinal center in a normal lymph node.

either differentiate into short-lived plasma cells through an immunoblast stage or differentiate into memory cells that populate follicular marginal zones or recirculate in the blood. Several B-cell lymphomas can be correlated with these stages of development (Fig. 7-8) and are mentioned in the sections that follow on separate lymphomas.

## ● PRACTICAL CONSIDERATIONS IN THE DIAGNOSIS OF LYMPHOMA

Determining a benign from malignant lymphoid infiltrate often can be difficult because malignant lymphocytes in many lymphomas closely resemble their benign counterparts. Therefore, diagnosis commonly rests on demonstrating a combination of an abnormal architectural pattern, an abnormal immunophenotype, and evidence of lymphoid monoclonality. As a result, several ancillary special studies have become

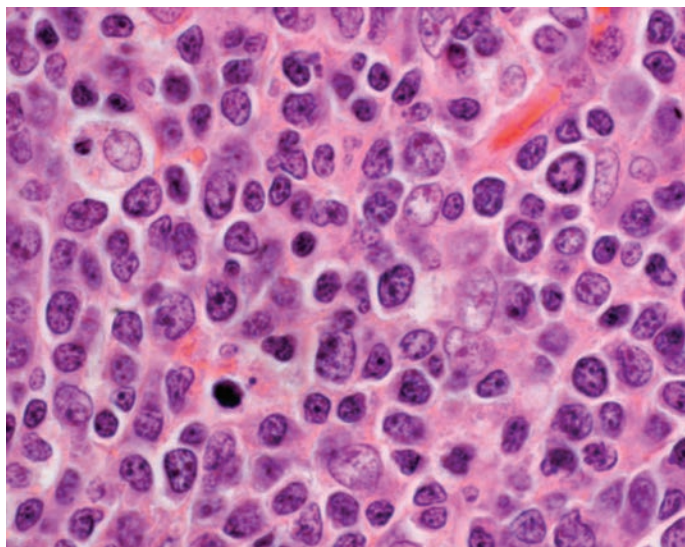


**Figure 7-7.** Spectrum of cells within the germinal center in a normal lymph node, ranging from small lymphocytes to larger cells with nucleoli.

instrumental in the diagnosis and classification of lymphoma, requiring special handling of the biopsy material (Table 7-1). Whenever a diagnosis of lymphoma is considered clinically, the surgeon should perform an open biopsy of the largest involved lymph node. The lymph node should be removed intact whenever possible because assessment of architecture is extremely important in the diagnosis and classification of lymphomas. The lymph node should be sent immediately to the pathology laboratory in the fresh state, at which time the pathologist allocates the tissue for fixation for routine histology and for special studies.

Automated flow cytometry on single-cell suspensions prepared from tissue samples is extremely helpful in demonstrating B-cell clonality by surface immunoglobulin light-chain restriction. It also determines the expression pattern of surface markers helpful in subclassifying lymphomas, particularly lymphomas of small B cells.<sup>17</sup> A wide variety of antibodies that can be used on formalin-fixed tissue now are available, allowing accurate diagnosis and subclassification of lymphoma in most cases.

Molecular genetic techniques to determine B- or T-cell monoclonality or lymphoma-specific chromosomal translocations include polymerase chain reaction (PCR), Southern blot, fluorescence *in situ* hybridization (FISH), and cytogenetic analysis.<sup>18</sup> PCR and FISH can be performed on formalin-fixed tissue. Results from molecular genetic testing should be interpreted in conjunction with the morphologic and immunophenotypic data, as some benign reactive lymphoid proliferations show evidence of lymphoid monoclonality.<sup>19</sup>

The diagnosis of lymphoma has become more complex than the diagnosis of other malignancies because diagnosis of lymphoma rests on correlation of morphologic features with immunophenotype and genetic data in many cases. Because of the complexity of diagnosis and the relative infrequency of lymphoma in general pathology practice, a second review by a hematopathologist with expertise in lymphoma pathology is recommended. The second review can have a significant impact on the clinical management of patients.<sup>20</sup>

Whereas open biopsy of an involved lymph node is the most useful diagnostic procedure, core-needle biopsies and fine-needle aspiration can play a role in limited situations. Core-needle biopsy might be helpful in the diagnosis of deep-seated disease in the abdomen, allowing the patient to avoid a laparotomy. However, a definitive diagnosis by core biopsy is not always possible, necessitating an open biopsy. Fine-needle aspiration is not helpful in primary diagnosis of lymphoma,<sup>21</sup> but it may be helpful in detecting recurrence of a previously diagnosed lymphoma or in ruling out a nonhematolymphoid lesion causing lymphadenopathy. Although automated flow cytometry can be used in conjunction with cytologic examination to provide additional information for lymphoma diagnosis and classification, tissue biopsy generally is required before commencement of therapy.

## ● PRECURSOR B- AND T-CELL LYMPHOMAS/LEUKEMIAS

Lymphoblastic leukemia/lymphoma represents a malignancy of lymphoblasts, of either B or T lineage. They can present in the marrow (leukemia) or with predominant tissue involvement (lymphoma), but they are considered single-disease entities. Most cases of acute lymphoblastic leukemia are of B lineage, whereas most cases of lymphoblastic lymphoma are of T lineage, with the mediastinum being a common site of involvement. The morphologic features are the same regardless of site or lineage, consisting of small- to intermediate-size cells with finely dispersed nuclear chromatin, inconspicuous nucleoli, and scant cytoplasm (Fig. 7-9). Assessment of lineage and distinction from minimally differentiated acute myeloid leukemia require immunophenotypic data and may require molecular genetic analysis of B- and T-cell receptors.



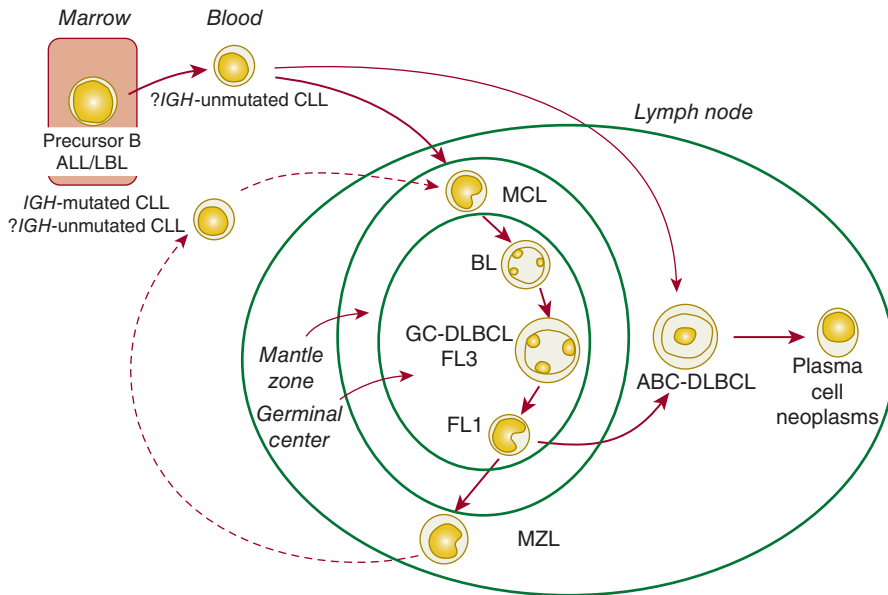


**Figure 7-8.** B-cell neoplasms correlate with different stages of development. ABC-DLBCL, activated B-cell type diffuse large B-cell lymphoma; ALL/LBL, acute lymphoblastic leukemia/lymphoblastic lymphoma; BL, Burkitt lymphoma; CLL, chronic lymphocytic leukemia; FL1, follicular lymphoma, grade 1; FL3, follicular lymphoma, grade 3; GC-DLBCL, germinal center-type diffuse large B-cell lymphoma; IGH, immunoglobulin heavy chain; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma.

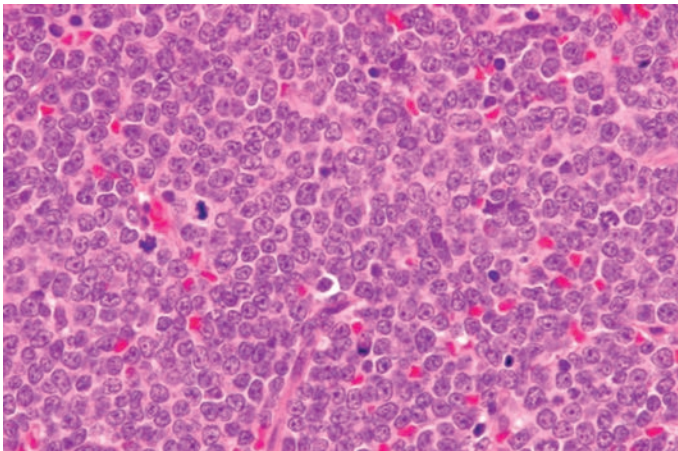


**Figure 7-9.** Lymphoblastic lymphoma of T-cell type, characterized by a diffuse proliferation of medium-size cells with finely distributed chromatin and high mitotic activity.

Lymphoblastic neoplasms are distinguished from other lymphomas by the expression of terminal deoxynucleotide transferase, which is specifically expressed at the lymphoblast stage of development.

The 2008 WHO classification included several categories of B-lymphoblastic leukemia/lymphoma characterized by recurrent genetic abnormalities.<sup>14</sup> Many of these are associated with distinct clinical or pathologic features, have prognostic implications, or are considered biologically distinct entities.

## ● MATURE B-CELL NON-HODGKIN LYMPHOMAS

### CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA

Chronic lymphocytic leukemia is a neoplasm of mature B lymphocytes characterized by blood and marrow involvement and commonly associated with lymph node involvement (Chap. 3). Small lymphocytic

lymphoma is the nonleukemic form of the disease. Lymph nodes involved by chronic lymphocytic leukemia show a diffuse infiltrate of small mature lymphocytes admixed with prolymphocytes and paraimmunoblasts, which characteristically form ill-defined nodules known as *proliferation* or *growth centers* (Figs. 7-10 and 7-11). The B cells have a characteristic immunophenotype, demonstrating CD5 and CD23 expression and dim expression of CD20 and clonal immunoglobulin light chain. Studies have divided chronic lymphocytic leukemia into two distinct subtypes with distinct clinical behavior (Chap. 3). The type with the more favorable prognosis expresses mutated variable regions of the immunoglobulin heavy-chain (*IGH*) genes, whereas the other subtype expresses unmutated *IGH* genes. The *IGH* gene mutation status is reflected in differences in gene expression.<sup>22,23</sup> The gene encoding the zeta-associated protein of 70 kDa (ZAP-70) is one of these genes, which generally is expressed by leukemia cells that express unmutated *IGH* genes and hence can be used to discriminate between the two subtypes.<sup>24</sup> Certain cytogenetic abnormalities also correlate with clinical aggressiveness.<sup>25</sup>

Some cases of chronic lymphocytic leukemia/small lymphocytic lymphoma demonstrate plasmacytic features but are distinct from an entity known as *lymphoplasmacytic lymphoma*, which is characterized

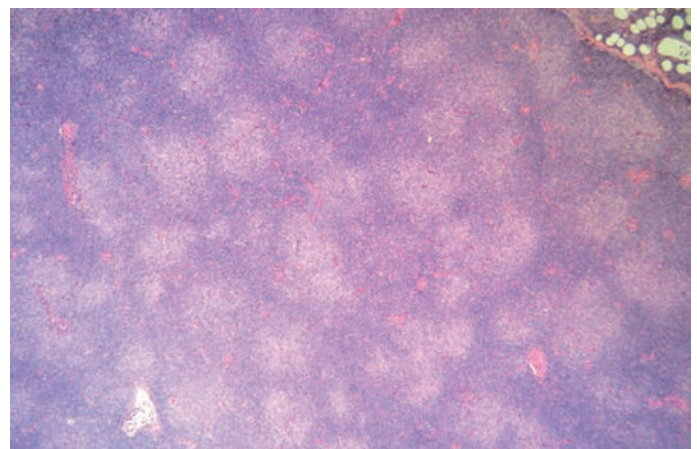


**Figure 7-10.** Small lymphocytic lymphoma with vague nodular appearance imparted by proliferation centers.



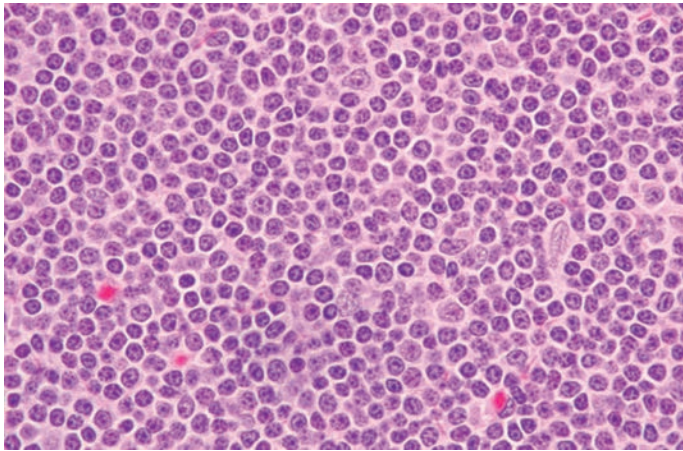


**Figure 7-11.** Small lymphocytic lymphoma characterized by small lymphocytes with mature chromatin pattern. Note that individual lymphocytes in small lymphocytic lymphoma are morphologically indistinguishable from benign lymphocytes.

by a prominent component of plasmacytic lymphocytes and plasma cells (Fig. 7-12). These cases typically do not express CD5, less often involve blood, and often are associated with a monoclonal immunoglobulin M serum protein that can cause hyperviscosity or cryoglobulinemia (Waldenström macroglobulinemia). Somatic mutations in *MYD88* are a commonly recurring and highly specific feature of Waldenström macroglobulinemia.<sup>26</sup>

## MANTLE CELL LYMPHOMA

Mantle cell lymphoma most commonly involves lymph nodes, but it can involve extranodal sites, including the gastrointestinal tract, as a clinical variant known as *lymphomatous polyposis* (Fig. 7-13). It typically is composed of a uniform population of small lymphocytes with cleaved nuclei and a virtual absence of large transformed cells (Fig. 7-14).<sup>27,28</sup> It most commonly has a diffuse growth pattern, but it can show a nodular or, more rarely, a mantle zone pattern (Fig. 7-15). The postulated cell of origin is the B cell of the inner mantle zone. The lymphoma cells coexpress CD5, as does chronic lymphocytic leukemia, but mantle cell lymphoma can be distinguished by lack of CD23 expression and expression

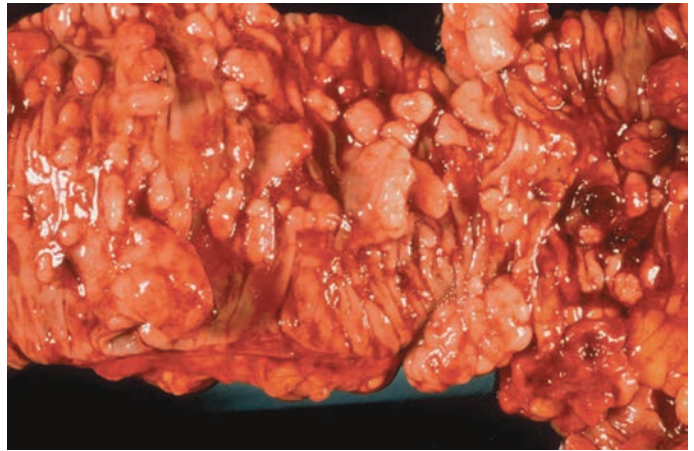


**Figure 7-13.** Large bowel involved with mantle cell lymphoma (multiple lymphomatous polyposis).

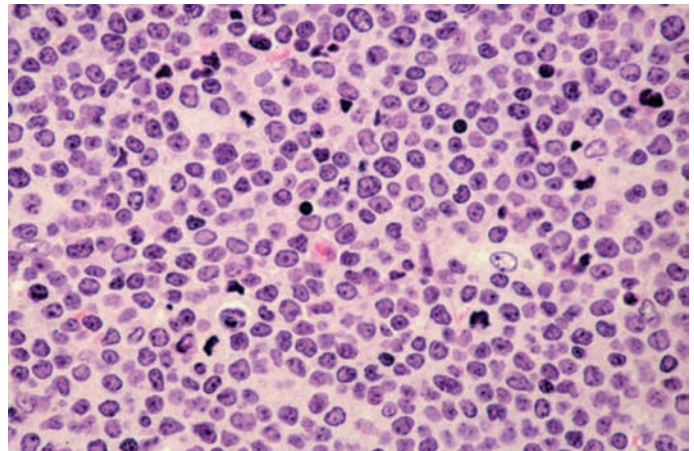


**Figure 7-14.** Mantle cell lymphoma with a diffuse pattern, characterized by a monomorphous infiltrate of small irregular lymphocytes with numerous mitotic figures.

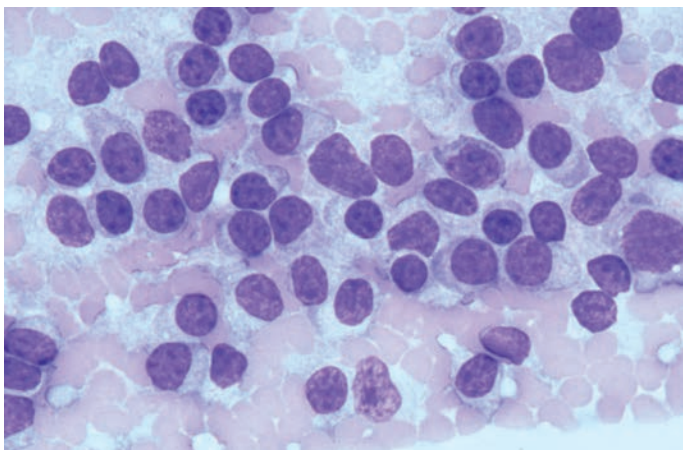


**Figure 7-12.** Imprint preparation of lymphoplasmacytic lymphoma demonstrating small lymphocytes and cells with plasmacytoid features (eccentric nuclei and bluish cytoplasm).

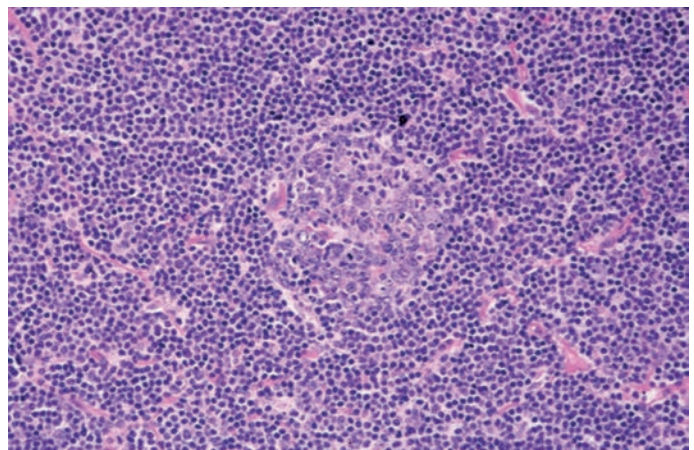


**Figure 7-15.** Mantle cell lymphoma with a mantle zone pattern, characterized by monomorphous small lymphocytes surrounding a benign germinal center.



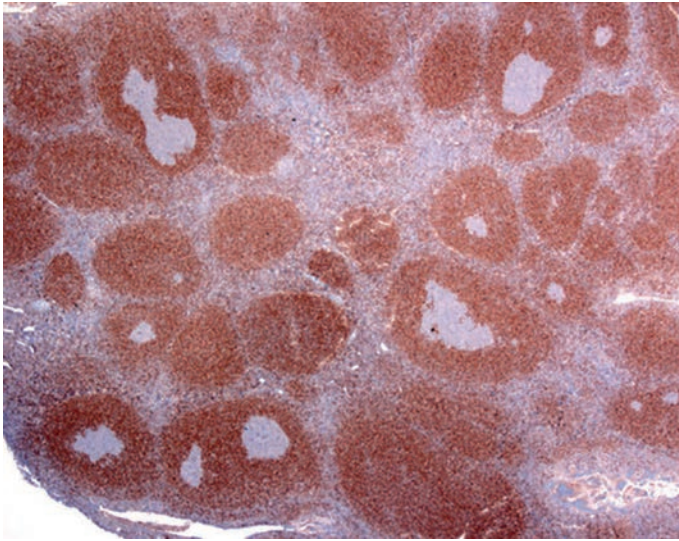


**Figure 7-16.** Mantle cell lymphoma with mantle zone pattern stained with antibody to cyclin D1.

of cyclin D1 (Fig. 7-16). Cyclin D1 expression results from the chromosomal translocation  $t(11;14)(q13;q32)$  characteristic of mantle cell lymphoma. Gene expression data have demonstrated a subset of mantle cell lymphomas that are cyclin D1 negative.<sup>29</sup> Some of these cyclin D1-negative cases have chromosomal translocations involving the cyclin D2 gene.<sup>30</sup> SOX11 expression is a highly specific immunohistochemical marker for mantle cell lymphoma and can identify cases that are negative for cyclin D1.<sup>31</sup> Overall, patients with mantle cell lymphoma have a median survival of approximately 3 years, but gene expression data that determine tumor cell proliferation are able to identify patient subsets that differ in median survival by more than 5 years<sup>29</sup> (Chap. 11).

Cyclin D1-positive lymphocytes in mantle zones can be an incidental finding in reactive lymphoid follicles, a condition referred to as *in situ* mantle cell lymphoma. These appear to have an indolent behavior and do not require treatment.<sup>32</sup>

## FOLLICULAR LYMPHOMA

Follicular lymphoma is a proliferation of cells that correspond to normal germinal center cells,<sup>33</sup> retaining expression of germinal center markers (BCL6, CD10), and demonstrates a follicular architecture (Fig. 7-17) imparted by nodular aggregates of CD21-positive follicular dendritic cells. Follicular lymphomas are composed of a variable mixture of centrocytes (small cleaved cells) and centroblasts (large noncleaved cells). They can be divided into three grades (grades 1 to 3) based on the number of centroblasts present. The most common is grade 1 (0–5 centroblasts per high-power microscopic field), previously known as *follicular small-cleaved-cell lymphoma* (Fig. 7-18). Both grade 1 and grade 2 tumors are indolent, and distinguishing between them is not required. Grade 3 follicular lymphoma (>15 centroblasts per high-power microscopic field) can be further divided into grade 3A (mixture of centroblasts and centrocytes) (Fig. 7-19) and grade 3B (solid sheets of centroblasts). Data have shown some molecular genetic differences between 3A and 3B cases, but further study is required because no significant clinical impact has been demonstrated.<sup>34,35</sup> Follicular lymphoma can have an accompanying diffuse component, and identification of a diffuse area of large cells (diffuse large B-cell lymphoma) indicates transformation to a more aggressive disease. Approximately 90 percent of follicular lymphoma demonstrate the  $t(14;18)(q32;q21)$  involving

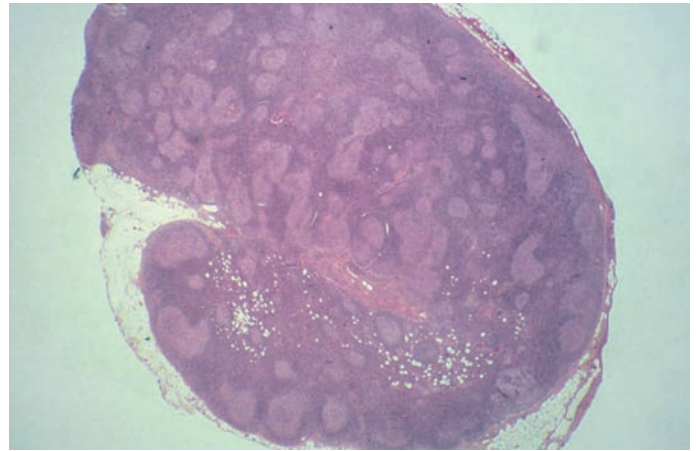


**Figure 7-17.** Grade 2 follicular lymphoma (low-power magnification), characterized by crowded follicles throughout the entire lymph node.

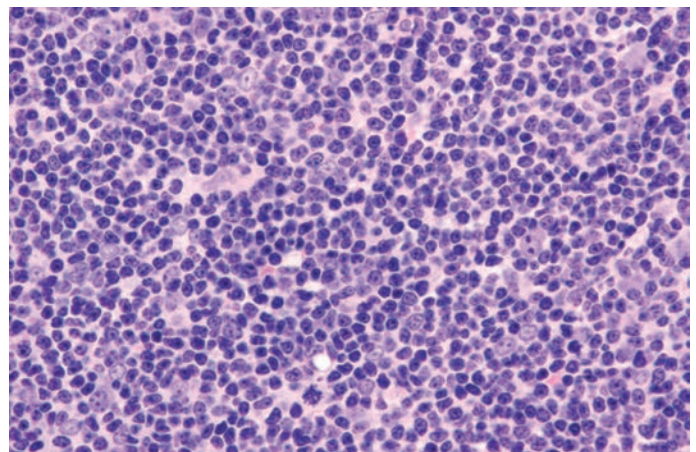


**Figure 7-18.** Center of a neoplastic follicle in grade 1 follicular lymphoma with almost exclusively small centrocytes.

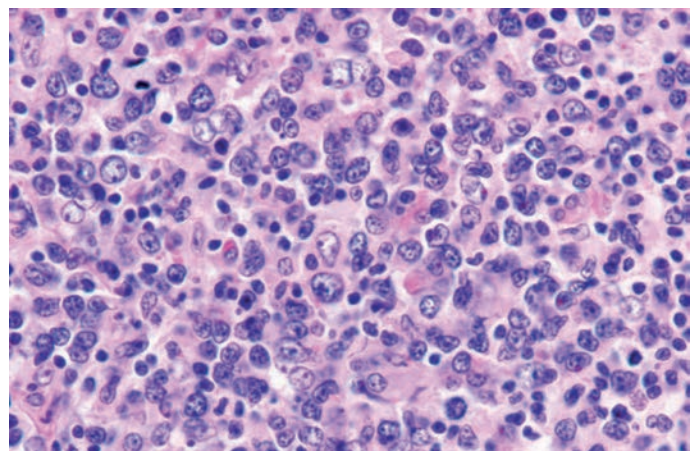


**Figure 7-19.** Grade 3A follicular lymphoma with >15 centroblasts per high-power field.



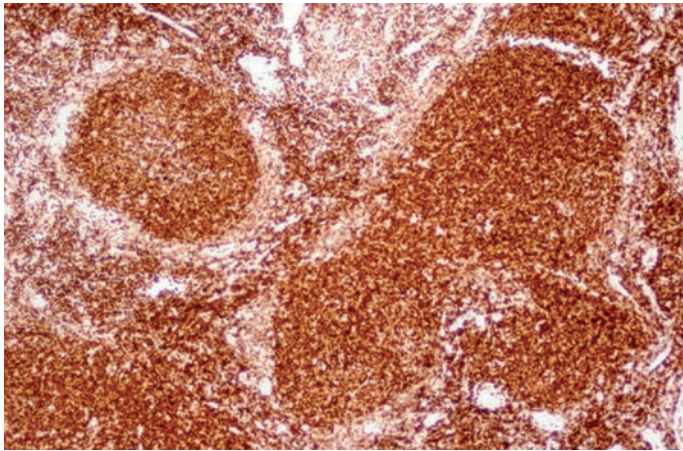


**Figure 7-20.** Positive BCL2 immunostain of a follicular lymphoma (contrast with Fig. 7-5).

rearrangement of the *BCL2* gene, leading to the constitutive expression of the antiapoptotic BCL2 protein. Although BCL2 protein expression does not help distinguish follicular lymphoma from other lymphomas, it is a helpful feature in distinguishing it from reactive follicles that are BCL2 negative (Fig. 7-20).

Follicular lymphoma *in situ* is an entity where BCL2-positive germinal centers are present in an otherwise reactive lymph node. When it is distinguished from partial involvement by follicular lymphoma, follicular lymphoma *in situ* has a very low rate of progression to overt follicular lymphoma.<sup>36</sup>

## MARGINAL ZONE B-CELL LYMPHOMAS

Marginal zone lymphomas are characterized by a proliferation of small lymphocytes, commonly with abundant pale cytoplasm (called *monocytoid B cells*) and plasmacytic features. The postulated cell of origin of these lymphomas is the postgerminal center B cell of the marginal zone at various anatomic sites. Marginal zone lymphomas can be divided into three distinct types based on site of presentation: (1) extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT), (2) splenic marginal zone lymphomas,<sup>37</sup> and (3) nodal marginal zone lymphomas (Fig. 7-21).<sup>38</sup> This classification is supported by distinctive

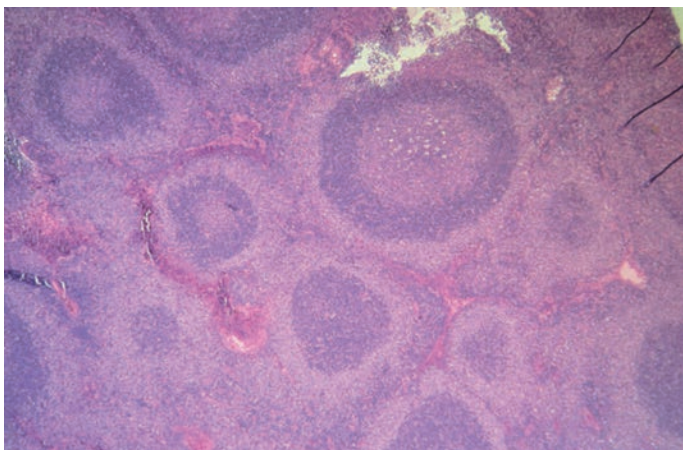


**Figure 7-21.** Lymph node involved by marginal zone B-cell lymphoma, in which the benign germinal centers and mantle zones are surrounded by expanded pale marginal zones.

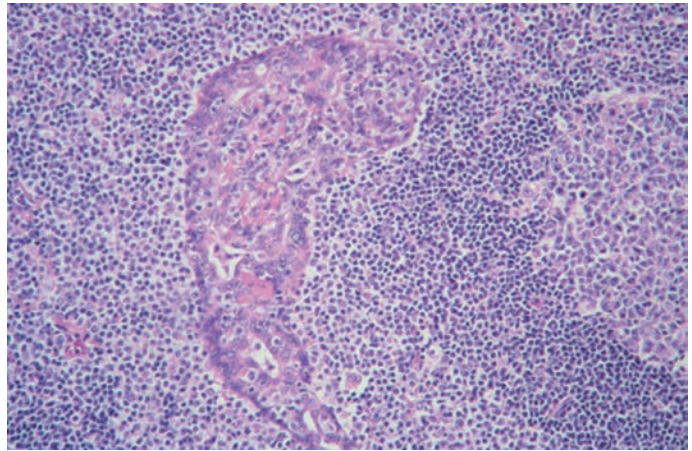


**Figure 7-22.** Salivary gland involved by mucosa-associated lymphoid tissue (MALT) lymphoma, showing a diffuse infiltrate of small lymphocytes with pale cytoplasm, infiltrating an enlarged salivary gland duct (lymphoepithelial lesion).

cytogenetic abnormalities in each entity. Extranodal lymphomas of the MALT type are the most common and arise in mucosal sites subject to long-standing chronic inflammation (Fig. 7-22), including chronic infection, the prototypical example being chronic *Helicobacter pylori* infection of the stomach.<sup>39</sup> At early stages of development, many of these lymphomas respond to treatment with antibiotics to eradicate *H. pylori*, whereas later changes, including cases with chromosomal translocations activating genes involved in nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling,<sup>40</sup> lead to antigen-independent growth (Chap. 12).

## DIFFUSE LARGE B-CELL LYMPHOMA

Diffuse large B-cell lymphoma (DLBCL) is characterized by a diffuse infiltrate of large B cells that can resemble centroblasts or immunoblasts (Figs. 7-23 and 7-24). The 2008 WHO classification identified several types of large B-cell lymphoma, the most common type being DLBCL not otherwise specified, which constitutes 25 to 30 percent of all non-Hodgkin lymphomas.

Gene-expression data show that DLBCL is a heterogeneous disease consisting of at least three entities having distinct gene-expression profiles based on cell of origin: (1) cases with an expression profile similar to germinal center B cells (GCBs), (2) cases expressing genes

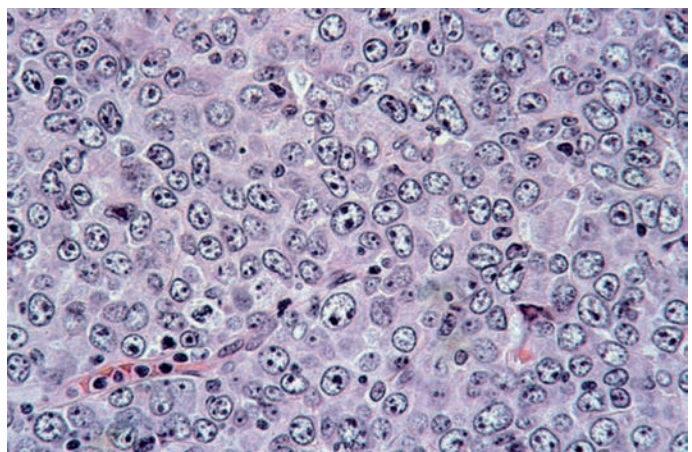


**Figure 7-23.** Diffuse large B-cell lymphoma.



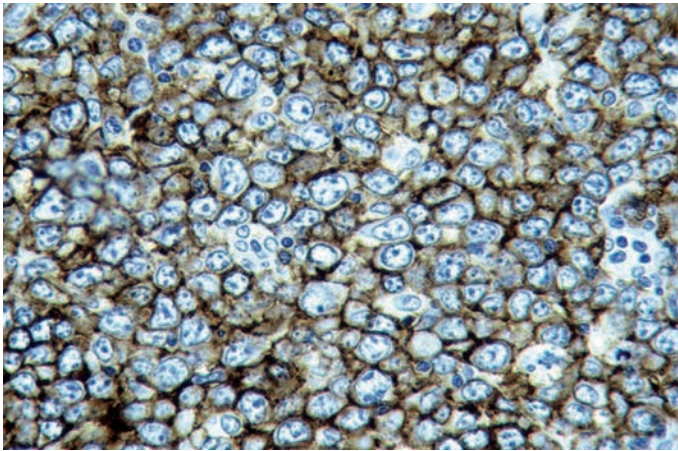


**Figure 7-24.** Diffuse large B cell stained with antibody to CD20 (B-cell marker).

typical of activated B cells (ABCs), and (3) cases with a different pattern referred to as “unclassifiable” that are neither GCB type nor ABC type (Fig. 7-25).<sup>41</sup> Importantly, clinical differences were apparent, with GCB-type cases having a significantly better prognosis compared to the other two types, even when clinical prognostic markers are considered (Chap. 9). Further studies confirmed these differences in the current era of therapy (including anti-CD20 antibody therapy), and identified nonneoplastic cells in the microenvironment as important contributors

to patient survival.<sup>42</sup> New therapies with selective activity in these subtypes of DLBCL are under development.<sup>43</sup> It has been suggested that division of DLBCL into clinically distinct groups may be determined by the expression profile of a limited number of genes using routine immunohistochemistry.<sup>44</sup> However, such an approach to classification is limited by problems of reproducibility of immunohistochemical staining and interpretation.<sup>45</sup> The study of gene expression patterns of a small number of genes using formalin-fixed, paraffin-embedded material may provide a rapid and accurate method to classify DLBCL.<sup>46</sup>

Rearrangement of the *MYC* gene is present in 5 to 10 percent of DLBCLs and is associated with an inferior prognosis.<sup>47</sup> Approximately half of these cases will also have a rearrangement involving the *BCL2* gene, referred to as a “double-hit” lymphoma. Such double-hit lymphomas have a very poor prognosis.<sup>48</sup> *MYC* protein expression is present in approximately 30 percent of DLBCLs, which may be independent of gene rearrangement. Concurrent expression of *MYC* and *BCL2* in DLBCL is associated with an inferior prognosis.<sup>49</sup>

Mediastinal large B-cell lymphoma is a distinct subtype of DLBCL that has been separately identified in the WHO classification.<sup>50</sup> Patients with mediastinal lymphomas typically are younger than those with conventional DLBCL. The histology shows large cells with abundant cytoplasm associated with diffuse fibrosis (Fig. 7-26). Gene expression studies have demonstrated an expression profile that is distinct from conventional diffuse large B-cell lymphoma and shares some features with classic Hodgkin lymphoma (Fig. 7-27).<sup>51,52</sup> Indeed, the 2008 WHO classification recognized that some cases of mediastinal lymphomas can have features that are intermediate between DLBCL and classical Hodgkin lymphoma.<sup>14</sup>

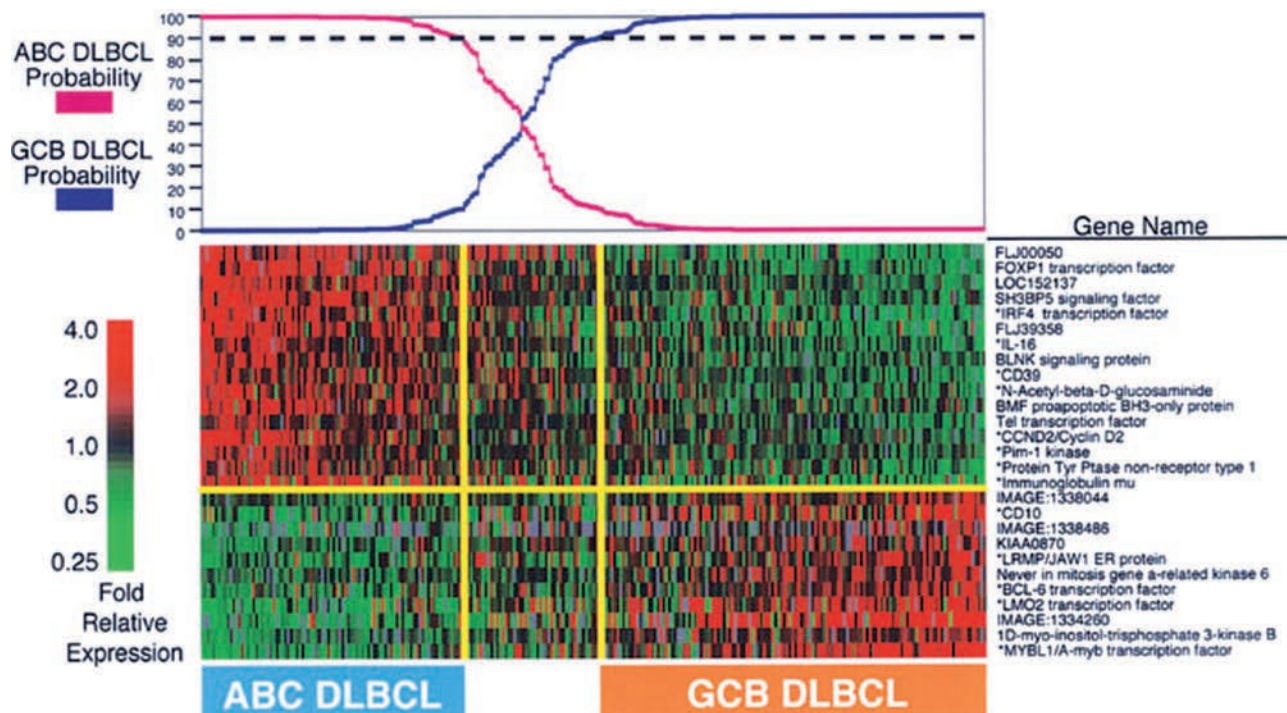


**Figure 7-25.** Gene-expression profiling diffuse large B-cell lymphoma (DLBCL) showing the subgroup discriminator used divides cases into germinal center B-cell-like (GCB) and activated B-cell-like (ABC). Each vertical column represents an individual patient and each horizontal row a unique gene. Red is relative overexpression of a gene and green relative underexpression. Using a probability of subgroup assignment of 90 percent, approximately 15 percent of cases are left unclassified (cases between the vertical yellow bars that are neither GCB nor ABC). This approach allows one to analyze thousands of genes from a single patient in one experiment and forms the basis of the new molecular classification of lymphoma. (Reproduced with permission from Wright G, Tan B, Rosenwald A, et al: A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma, *Proc Natl Acad Sci U S A* 2003 Aug 19;100(17):9991–9996.)



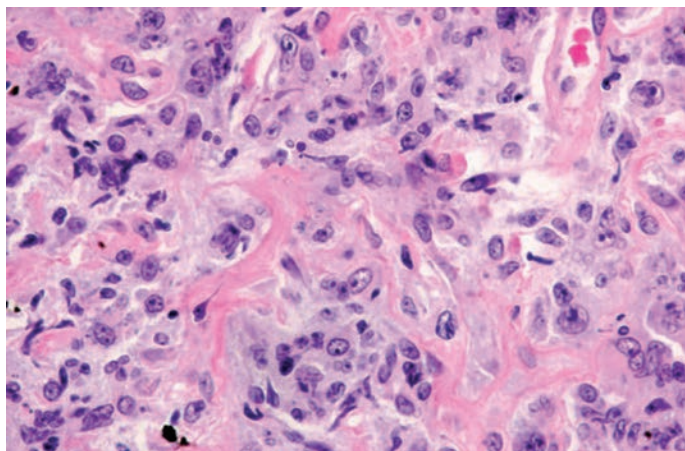


**Figure 7-26.** Primary mediastinal large B-cell lymphoma with sclerosis.

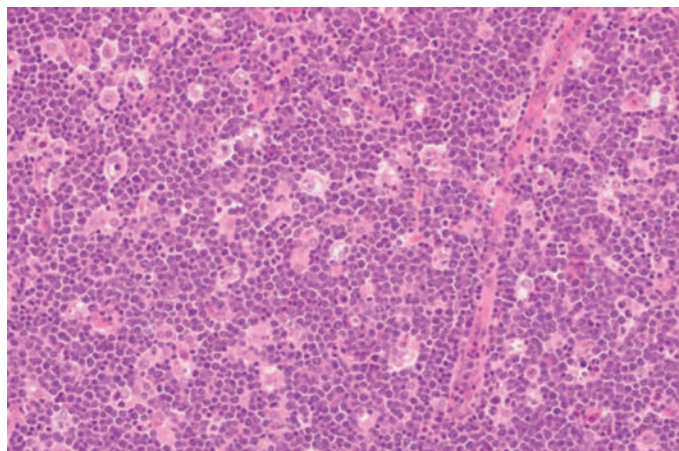


**Figure 7-28.** Burkitt lymphoma with starry sky appearance, imparted by macrophages that have engulfed apoptotic debris of dying tumor cells.

## BURKITT LYMPHOMA

Burkitt lymphoma is a highly aggressive lymphoma characterized histologically by a diffuse infiltrate of intermediate-size cells with a high mitotic rate. The lymphomas commonly have significant spontaneous

cell death (apoptosis), which results in a “starry sky” appearance caused by numerous macrophages that have engulfed the apoptotic debris (known as *tingible body macrophages*) (Figs. 7-28 and 7-29). The postulated cell of origin is the early follicular B blast cell of the germinal center.

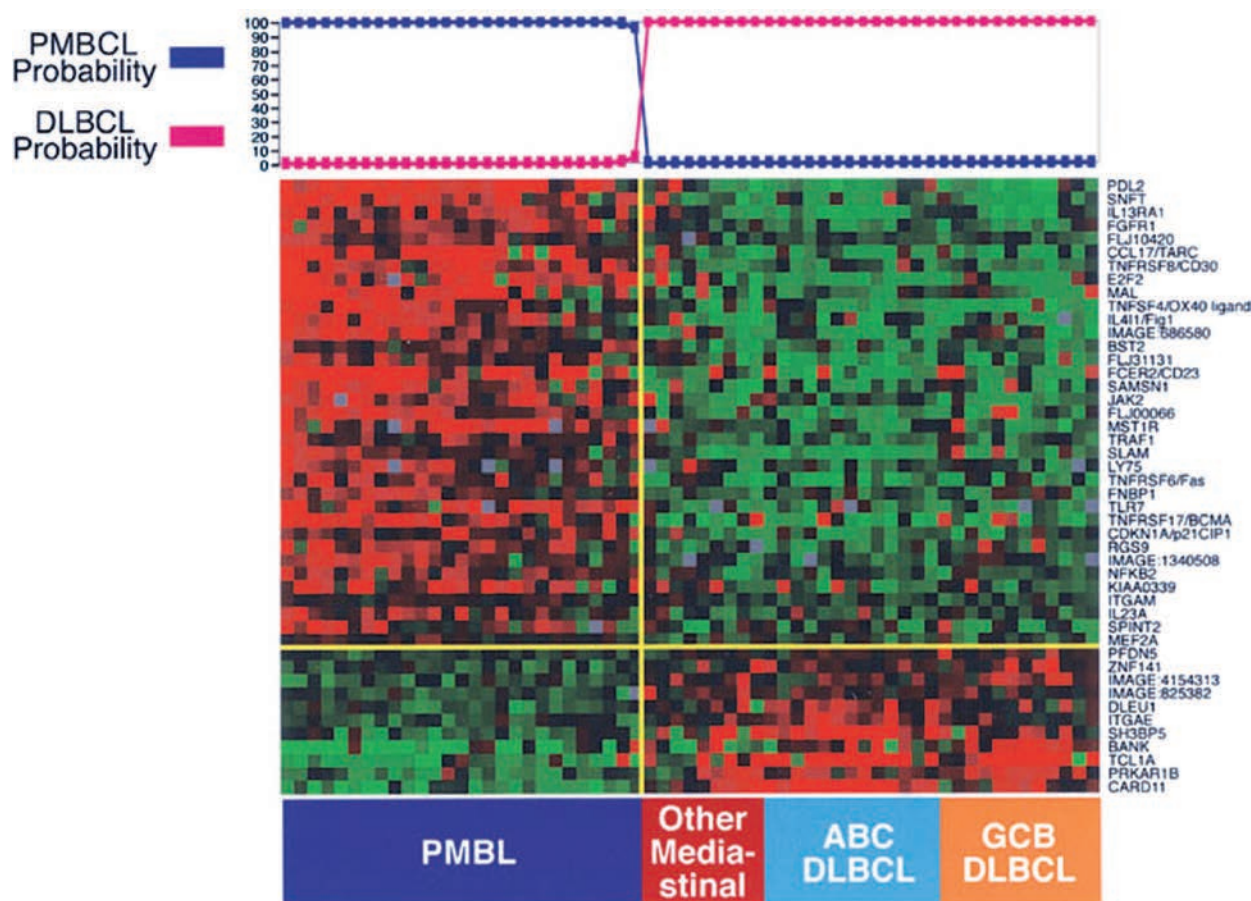


**Figure 7-27.** Gene-expression profiling of primary mediastinal large B-cell lymphoma (PMBCL), contrasting the expression profile with nodal diffuse large B-cell lymphomas (DLBCL). This figure shows numerous genes that are overexpressed in PMBCL (red). Many of these genes are shared with classical Hodgkin lymphoma, suggesting a biologic overlap between these two diseases. Cases listed as “Other Mediastinal” refer to those cases of DLBCL with mediastinal involvement but not felt to be typical of PMBCL. This is borne out by the gene-expression data, showing that these cases are more closely related to DLBCL rather than PMBCL. ABC, activated B cell; GCB, germinal center B cell. (Reproduced with permission from Rosenwald A, Wright G, Leroy K, Yu X, et al: *Molecular Diagnosis of Primary Mediastinal B Cell Lymphoma Identifies a Clinically Favorable Subgroup of Diffuse Large B Cell Lymphoma Related to Hodgkin Lymphoma*, J Exp Med 2003 Sep 15;198(6):851–862.)



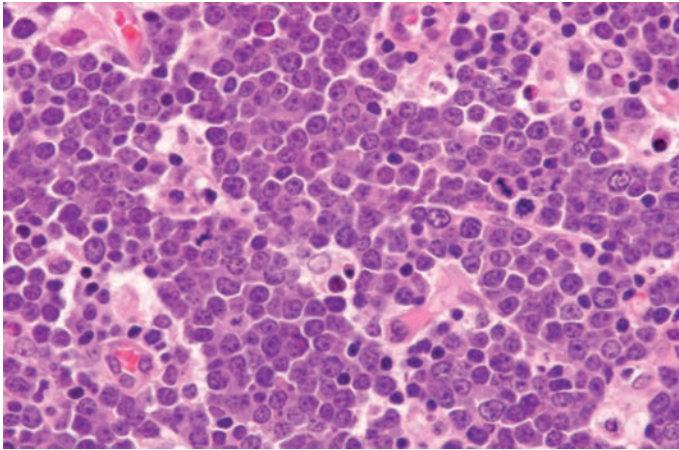


**Figure 7-29.** Burkitt lymphoma, characterized by a diffuse infiltrate of medium-size cells with small nucleoli and a high mitotic activity.

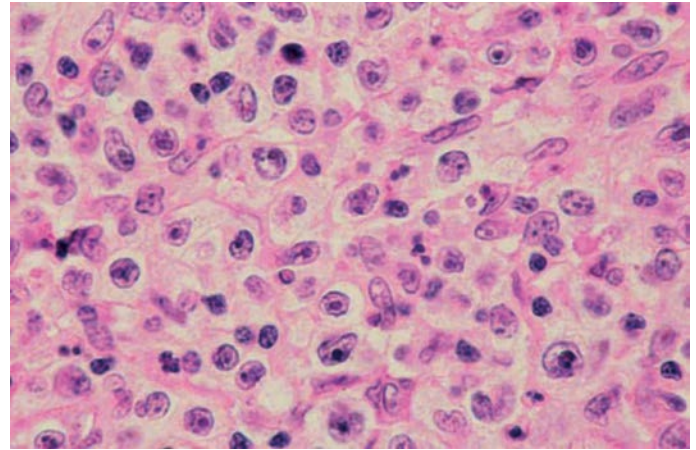


**Figure 7-30.** Peripheral T-cell lymphoma, unspecified, composed predominantly of large cells.

Virtually all cases of Burkitt lymphoma are characterized by chromosomal translocations involving the *MYC* gene on chromosome 8. The *MYC* gene most commonly is translocated to the *IGH* gene on chromosome 14, resulting in t(8;14)(q24;q32), but it also can involve the light-chain genes on chromosomes 2p12 ( $\kappa$ ) and 22q11 ( $\lambda$ ). A diagnosis of Burkitt lymphoma can be suggested based on morphologic examination alone but should be supported by immunophenotypic data (positive for CD20, CD10, and BCL6; negative or focally weakly positive for BCL2; growth fraction near 100 percent as determined by Ki67 stain) and confirmed by molecular testing for *MYC* translocations whenever possible.

Gene-expression studies have shown that Burkitt lymphoma has a consistent gene-expression signature, but that there is not always correlation between the diagnosis based on gene-expression profiling and the diagnosis based on standard diagnostic testing.<sup>53,54</sup> To reflect this, the 2008 WHO classification recognized a provisional entity of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma.<sup>14</sup> In the 2016 WHO revision, such cases are now divided into two groups of high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements. The remaining cases are classified as high-grade B-cell lymphoma not otherwise specified.<sup>14a</sup>

## ● MATURE T-CELL AND NK-CELL NON-HODGKIN LYMPHOMAS

T cells and NK cells share several immunophenotypic and functional features; therefore, these neoplasms are grouped together in the WHO classification. These lymphomas make up 10 to 15 percent of non-Hodgkin lymphomas in Western countries, with a higher incidence in Asia. Mature T-cell lymphomas comprise a heterogeneous group of neoplasms, the most common subtype being the peripheral T-cell lymphoma (PTCL) not otherwise specified.

PTCLs typically grow in a diffuse pattern that effaces normal nodal architecture or, more rarely, show expansion of the interfollicular areas. They show a diverse cytologic spectrum, with most cases showing a mixture of large- to intermediate-size cells and occasional cases showing predominantly small cells (Figs. 7-30 and 7-31). Cell type has no prognostic relevance. A reactive background consisting of eosinophils, plasma cells, and macrophages may be present, in which

case the diagnosis of Hodgkin lymphoma may be entertained. Immunophenotypic data cannot prove clonality as in B-cell lymphomas, but evidence of an aberrant T-cell phenotype supports a diagnosis of T-cell lymphoma. Molecular techniques to demonstrate clonal rearrangement of T-cell receptor genes can be helpful in confirming the diagnosis. Gene-expression profiling has helped to delineate biologic and prognostic groups within PTCL not otherwise specified.<sup>55</sup> Angioimmunoblastic T-cell lymphoma is a mature T-cell lymphoma that typically presents with systemic symptoms and polyclonal hypergammaglobulinemia and arises from a distinct subset of helper T cells, the follicular helper T cell.<sup>56</sup>

Anaplastic large cell lymphoma (ALCL) represents a unique subtype of T-cell lymphoma, particularly common in children. ALCL can show significant morphologic variability but typically is composed of large pleomorphic cells characterized by the presence of “hallmark” cells with horseshoe- or kidney-shaped nuclei and a perinuclear eosinophilic region (Fig. 7-32).<sup>57</sup> Partial involvement of lymph nodes can be limited to the sinuses, with obliteration of nodal architecture in later stages. ALCL is characterized by uniform, strong expression of CD30 (Fig. 7-33). The majority of cases express one or more T-cell antigens and demonstrate clonal T-cell receptor gene rearrangement.<sup>57</sup> ALCL is divided into two entities based on the expression of anaplastic lymphoma kinase (ALK) (Fig. 7-34). ALK-positive ALCL is most

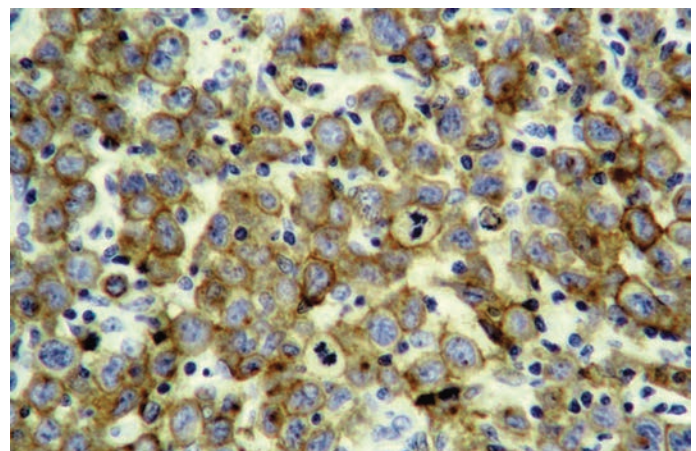


**Figure 7-31.** Peripheral T-cell lymphoma stained with antibody to CD3 (T-cell marker).



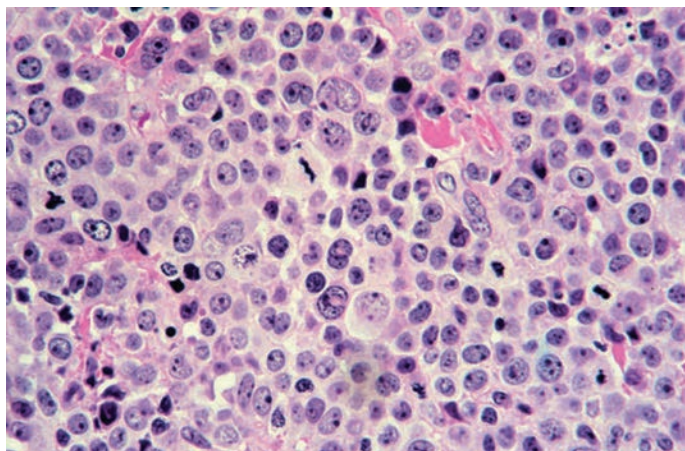


**Figure 7-32.** Anaplastic large cell lymphoma, T-cell type, containing a population of large cells with wreath-shaped nuclei and an eosinophilic perinuclear accentuation.

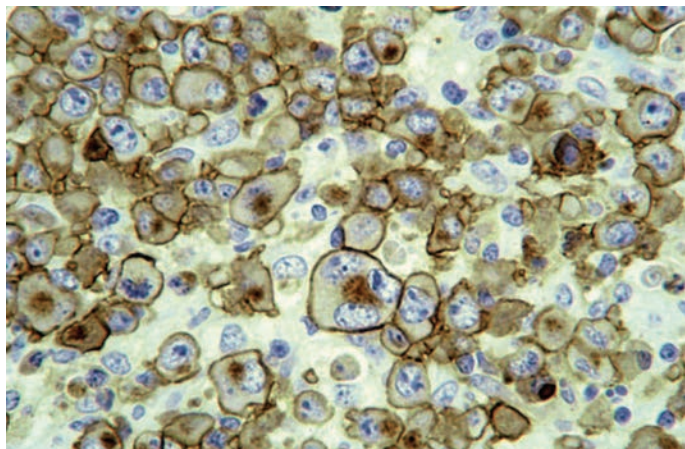


**Figure 7-33.** Anaplastic large cell lymphoma stained with antibody to CD30.

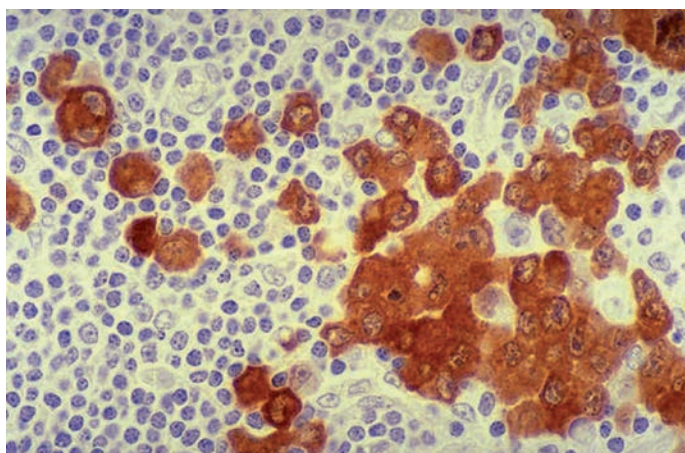


**Figure 7-34.** Anaplastic large cell lymphoma stained with antibody to ALK (anaplastic lymphoma kinase).

often seen in the first three decades of life and has a favorable prognosis compared to ALK-negative ALCL.<sup>58,59</sup> Expression of ALK is the result of chromosomal translocations involving the *ALK* gene on chromosome 2p23, the most common translocation being the t(2;5)(p23;q35) involving the nucleophosmin gene on chromosome 5.<sup>60</sup> ALK-negative ALCL is recognized as a provisional entity that is distinct from ALK-positive ALCL and PTCL, not otherwise specified.<sup>61</sup> Recently, additional genetic abnormalities have been identified in ALK-negative ALCL, involving *DUSP22* and *TP63* genes.<sup>62,63</sup>

Other types of mature T-/NK-cell lymphomas are uncommon and include enteropathy-associated T-cell lymphoma (an aggressive T-cell lymphoma typically arising in the small bowel from a background of celiac disease) and extranodal NK-/T-cell lymphoma, nasal type (an aggressive Epstein-Barr virus [EBV]-associated neoplasm commonly involving the nasal cavity). A detailed description of these specific lymphoma subtypes is beyond the scope of this chapter and can be found in the WHO classification.<sup>14</sup>

## ● HODGKIN LYMPHOMA

Hodgkin lymphoma consists of two distinct clinicopathologic entities: *classical Hodgkin lymphoma* (including four subtypes) and *nodular lymphocyte-predominant (LP) Hodgkin lymphoma* (Chap. 8).

### CLASSICAL HODGKIN LYMPHOMA

The neoplastic cell of classical Hodgkin lymphoma is the Reed-Sternberg cell, first described more than 100 years ago.<sup>64,65</sup> It is a large cell with two or more nuclei or nuclear lobes, each of which contains a large eosinophilic nucleolus (Fig. 7-35). The presence of Reed-Sternberg cells alone is insufficient for a diagnosis of Hodgkin lymphoma because cells with similar morphology can be seen in a variety of non-Hodgkin lymphomas and benign reactive conditions.<sup>66</sup> For a diagnosis of Hodgkin lymphoma, diagnostic Reed-Sternberg cells must be found in an appropriate background consisting of a variable polymorphous reactive infiltrate of inflammatory and accessory cells.<sup>67</sup>

Reed-Sternberg cells are derived from B cells in the vast majority of cases of classical Hodgkin lymphoma, as determined by clonal rearrangement of *IGH* genes.<sup>68</sup> However, Reed-Sternberg cells have lost most of their B-lineage antigens, including expression of immunoglobulin.

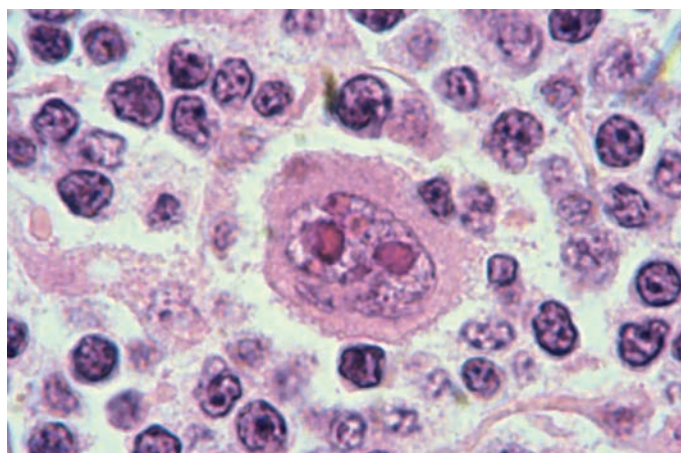


**Figure 7-35.** Diagnostic Reed-Sternberg cell in Hodgkin lymphoma.



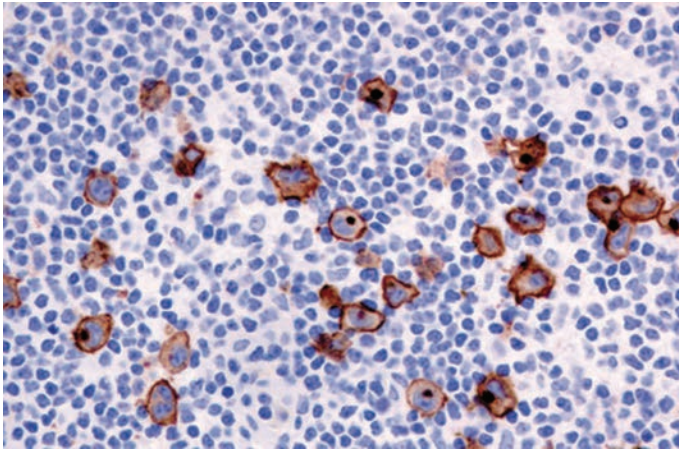


**Figure 7-36.** Classical Hodgkin lymphoma stained with antibody to CD30.

Reed-Sternberg cells express CD30 in almost all cases of classical Hodgkin lymphoma and express CD15 in the majority (Figs. 7-36 and 7-37).<sup>67</sup> They typically are negative for CD45 (leukocyte common antigen) and positive for B-cell marker CD20 in 20 to 40 percent of cases, usually of variable intensity in a minority of cells. Classical Hodgkin lymphoma is associated with EBV in 20 to 40 percent of cases and is thought to play a role in the pathogenesis of these cases.<sup>69</sup> Reed-Sternberg cells express many cytokines and several members of the tumor necrosis factor receptor family (e.g., CD40, CD30).<sup>70</sup> The cytokines are thought to play a role in the recruitment of reactive infiltrate and to contribute to Reed-Sternberg cell proliferation and survival. The tumor necrosis factor receptor family members can be activated by ligands expressed by the surrounding reactive infiltrate, leading to proliferation and survival.

The most common subtype of classical Hodgkin lymphoma is the nodular sclerosis variant. The variant is characterized by the presence of broad collagen bands dividing the tumor into nodules and by the presence of “lacunar” cells, mononuclear Reed-Sternberg variants that typically show retraction artifact so that the cells appear to be in lacunae (Fig. 7-38). These cells are found within a reactive infiltrate that typically includes prominent eosinophils and lymphocytes.

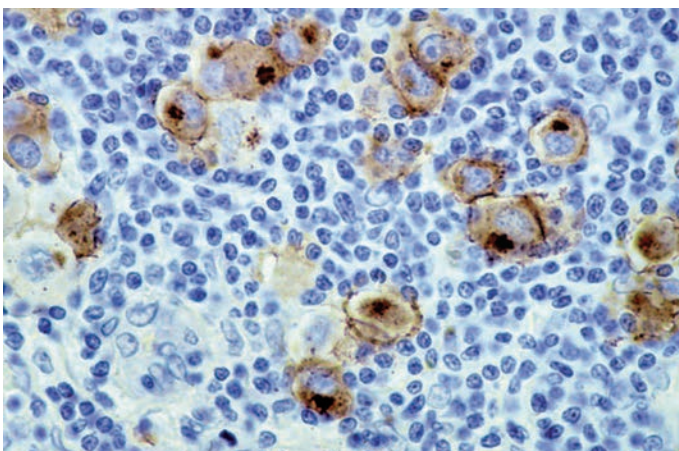


**Figure 7-37.** Classical Hodgkin lymphoma with Reed-Sternberg cells clearly identified with antibody to CD15.

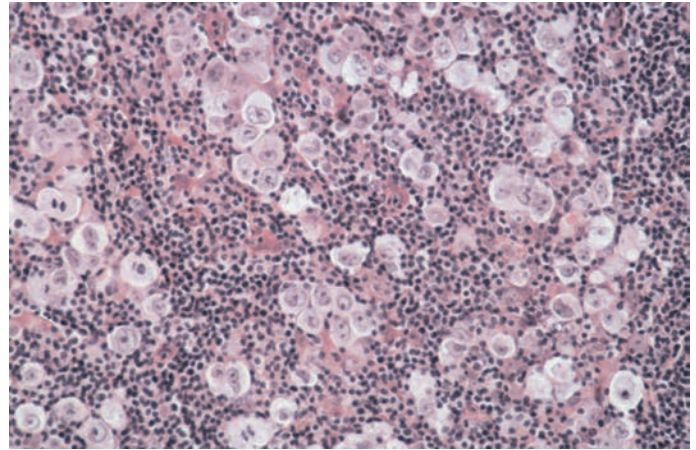


**Figure 7-38.** Classical Hodgkin lymphoma, nodular sclerosis type, with characteristic lacunar cells.

The second most common subtype is the mixed cellularity variant, which is characterized by Reed-Sternberg cells in a mixed inflammatory background without the broad collagen bands seen in nodular sclerosis (Fig. 7-39). Mixed cellularity cases are more commonly associated with EBV compared to the nodular sclerosis variant.

The lymphocyte-rich and lymphocyte-depleted subtypes of classical Hodgkin lymphoma are the least common, each representing approximately 5 percent of all cases. The lymphocyte-rich variant has a small number of Reed-Sternberg cells in a background of small lymphocytes with absent or rare eosinophils and neutrophils, typically in a nodular pattern. It is easily confused with nodular LP Hodgkin lymphoma, so immunohistochemical stains to determine the immunophenotype of the Reed-Sternberg cells is required to make the distinction.<sup>71,72</sup> It can rarely have a diffuse growth pattern.

In the past, the lymphocyte-depleted variant had been divided into reticular and diffuse fibrosis types. The diffuse fibrosis variant is characterized by a hypocellular infiltrate with prominent diffuse nonbirefringent sclerosis accompanied by rare Reed-Sternberg cells and a minor reactive inflammatory component. The reticular variant showed an increased number of large atypical cells, commonly with bizarre multinucleated cells, with a minor reactive component. It now is recognized

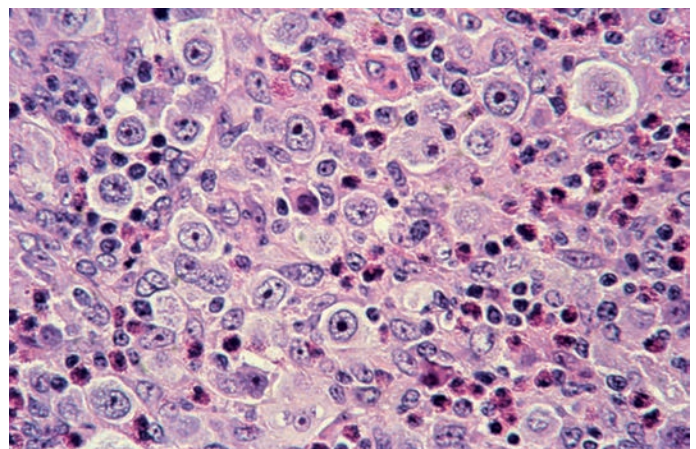


**Figure 7-39.** Mixed cellularity Hodgkin lymphoma.



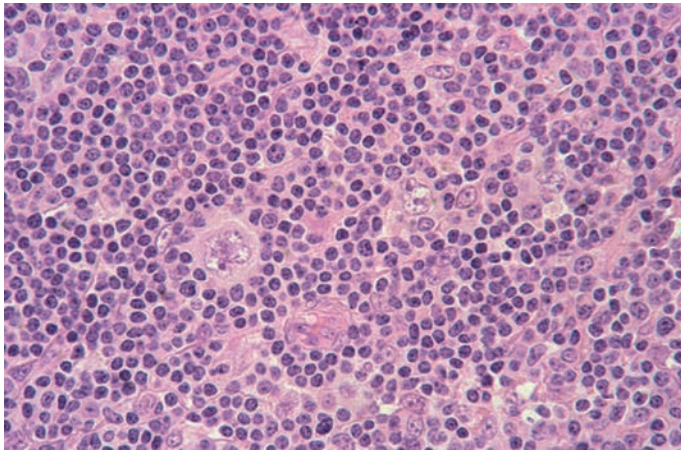


**Figure 7-40.** Nodular lymphocyte predominance Hodgkin lymphoma showing characteristic lymphocyte-predominant (LP) cells (popcorn cells) in a background of small benign lymphocytes.

that the vast majority of these cases are cases of ALCL or DLBCL, and as such the diagnosis of the reticular variant of lymphocyte-depleted Hodgkin lymphoma is rare and should be made only in the presence of definitive supportive immunophenotypic data.

## NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

Nodular LP Hodgkin lymphoma has several pathologic and clinical features that are distinct from classical Hodgkin lymphoma.<sup>73</sup> The malignant cells are known as LP cells, large cells with a single nucleus that contains multilobated or folded features. They often are referred to as “popcorn” cells because they resemble popped kernels of corn (Fig. 7-40). Nucleoli typically are smaller than the nucleoli seen in classical Reed-Sternberg cells. They differ from Reed-Sternberg cells in classical Hodgkin lymphoma in that they retain expression of CD45 and B-lineage markers (CD20, immunoglobulin) and are negative for CD15 and CD30 (Fig. 7-41).<sup>74</sup> As the name implies, the cells have a complete or partial nodular architectural pattern with a background consisting primarily of lymphocytes. Histiocytes are also a common feature, but neutrophils and eosinophils are absent or rare.

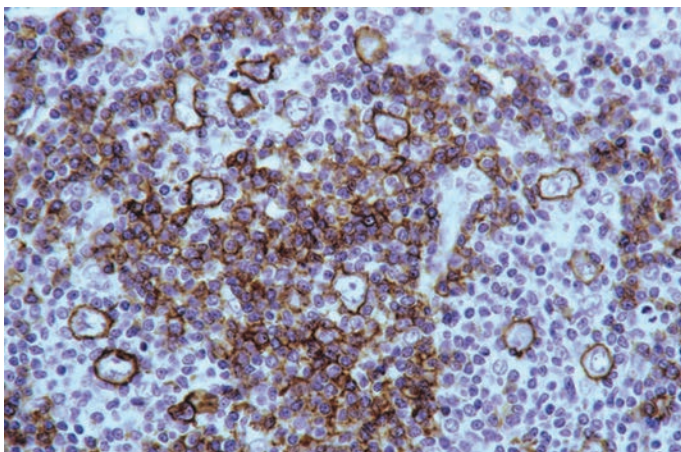


**Figure 7-41.** Nodular lymphocyte-predominant Hodgkin lymphoma stained with antibody CD20. Note the positive staining of the lymphocyte-predominant (LP) cells (popcorn cells).

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## CHAPTER 8

# HODGKIN LYMPHOMA

Oliver W. Press\* and John P. Leonard

### SUMMARY

Classical Hodgkin lymphoma is derived by malignant transformation of a mature B cell at the germinal center stage of differentiation and is characterized pathologically by multinucleated Hodgkin and Reed-Sternberg cells embedded in a mixed infiltrate of nonneoplastic cells. Hodgkin and Reed-Sternberg cells contain monoclonal immunoglobulin gene rearrangements but have lost most of the B-cell–specific expression program. Multiple signaling pathways and transcription factors are deregulated in Hodgkin lymphoma, and genetic lesions involving the JAK-STAT and nuclear factor- $\kappa$ B pathways are commonly identified. Epstein-Barr virus is an important environmental factor in the pathogenesis of Hodgkin lymphoma and also leads to activation of the nuclear factor- $\kappa$ B pathway. The inflammatory microenvironment promotes survival and allows escape of Hodgkin and Reed-Sternberg cells from immune attack. Morphologic and immunophenotypic features distinguish the four subtypes of classical Hodgkin lymphoma (accounting for 95 percent of cases) from nodular lymphocyte predominance Hodgkin lymphoma (accounting for 5 percent of cases). Hodgkin lymphoma spreads in a predictable, contiguous manner and is classified into four stages, I to IV. Classical Hodgkin lymphoma

**Acronyms and Abbreviations:** ABVD, Adriamycin (doxorubicin), bleomycin, vinblastine, dacarbazine; AP1, activator protein 1; BCMA, B-cell maturation antigen; BEACOPP, bleomycin, etoposide, Adriamycin (doxorubicin), cyclophosphamide, vincristine, procarbazine, prednisone; BEAM, bischloroethylnitrosourea (carmustine), etoposide, Ara C (cytarabine), melphalan; CBV, cyclophosphamide, bischloroethylnitrosourea (carmustine), etoposide; cHL, classical Hodgkin lymphoma; COPP, cyclophosphamide, vincristine, procarbazine, prednisone; CT, computed tomography; DHAP, dexamethasone, cytarabine, cisplatin; EBV, Epstein-Barr virus; EBVP, epirubicin, bleomycin, vinblastine, prednisone; EORTC, European Organization for the Research and Treatment of Cancer; ERK, extracellular signal-regulated kinase; ESR, erythrocyte sedimentation rate; FDG, 18-fluorodeoxyglucose; FIL, Fondazione Italiana Linfomi; GELA, Groupe d'Etude des Lymphomes de l'Adulte; GHSG, German Hodgkin Study Group; GVD, gemcitabine, vinorelbine, liposomal doxorubicin; HLA, human leukocyte antigen; ICE, ifosfamide, carboplatin, etoposide; IL, interleukin; INRT, involved nodal radiation therapy; JAK, Janus kinase; LMP, latent membrane protein; LySA, Lymphoma Study Association; MOPP, mechlorethamine (nitrogen mustard), Oncovin (vincristine), procarbazine, prednisone; NF- $\kappa$ B, nuclear factor- $\kappa$ B; NLPHL, nodular lymphocyte-predominant Hodgkin lymphoma; OS, overall survival; PD-1, cell death protein 1; PET, positron emission tomography; PI3K, phosphoinositide 3'-kinase; RANK, receptor activator of nuclear factor- $\kappa$ B; R-CHOP, rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine (Oncovin), prednisone; STAT, signal transducer and activator of transcription; TACI, transmembrane activator, calcium modulator, and cyclophilin ligand interactor.

is treated with the intent to cure the disease in all stages, and long-term survival exceeds 85 percent. Doxorubicin-containing chemotherapy plays a major role in treatment of all stages of the disease, whereas radiotherapy is used selectively because of concerns for late toxicities. 18-Fluorodeoxyglucose positron emission tomography is a valuable diagnostic test for assessment of disease extent and response to treatment. High-dose therapy and autologous transplantation are effective in patients who have relapsed, and several new and effective biologic agents are available, including brentuximab vedotin (an anti-CD30 antibody–drug conjugate) as well as nivolumab and pembrolizumab (anti-PD-1–blocking antibodies). Concerns regarding late treatment effects guide therapy and followup decisions in Hodgkin lymphoma, which disproportionately affects adolescents and young adults. Major treatment challenges include the maintenance of high cure rates with fewer short-term and long-term complications, biomarker identification of the small refractory subgroup, and integration of biologic therapies into treatment paradigms.

### DEFINITION AND HISTORY

Classical Hodgkin lymphoma (cHL) is a neoplasm of lymphoid tissue, in most cases derived from a germinal center B cell, defined by the presence of the malignant Hodgkin and Reed-Sternberg cells with a characteristic immunophenotype and appropriate cellular background. cHL accounts for 95 percent of cases and contains four histologic subtypes distinguished on the basis of microscopic appearance and relative proportions of Hodgkin and Reed-Sternberg cells, lymphocytes, and fibrosis (nodular sclerosis, mixed cellularity, lymphocyte rich, and lymphocyte depleted; [Table 8–1](#)). Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) represents the other major category, which is distinguished by Hodgkin and Reed-Sternberg variants termed *lymphocytic (L)* and *histiocytic cells (H)* that, unlike cHL, express typical B-lineage markers.

### HISTORICAL ASPECTS

In his historic 1832 paper, “On Some Morbid Appearances of the Absorbent Glands and Spleen,” Thomas Hodgkin described the clinical histories and gross postmortem findings of seven cases of the disease that was later to bear his name.<sup>1</sup> In 1856, Samuel Wilks independently described 10 cases of “a peculiar enlargement of the lymphatic glands frequently associated with disease of the spleen,” including four of Hodgkin’s original cases.<sup>2</sup> Upon discovering Hodgkin’s original report, he used the appellation “Hodgkin’s Disease” in a subsequent series of 15 cases published in 1865.<sup>2</sup> Thirteen years after Hodgkin’s original paper, the first cases of leukemia were described. Cases in which the neoplastic cells remained confined to the lymphatic system were described by Dreschfield (1892)<sup>3</sup> and Kundrat (1893)<sup>4</sup>; Kundrat gave the name *lymphosarcoma* to these cases. The description of additional members of the lymphoma-leukemia complex continued up to the present time.

Carl Sternberg (1898)<sup>5</sup> and Dorothy Reed (1902)<sup>6</sup> are credited with the first definitive and thorough descriptions of the pathology of cHL, although a number of investigators from England, Germany, and France had previously recognized the characteristic multinucleated giant cells. In 1926, Fox examined microscopic sections from the gross specimens of three of Hodgkin’s original cases preserved in the Gordon Museum of Guy’s Hospital in London.<sup>7</sup> It is remarkable that the preserved microanatomy allowed him to confirm the histopathologic diagnosis in two of these cases. Jackson and Parker made the first serious effort at the histopathologic classification of cHL, correlating their findings with

\*Sandra J. Horning, MD, was the author of this chapter for the eighth edition of Williams Hematology, and significant portions of that chapter have been retained.



**TABLE 8-1.** Classification of Hodgkin Lymphoma

| Histologic Subtype             | Immunophenotype        |
|--------------------------------|------------------------|
| Nodular lymphocyte predominant | CD20+ CD30– CD15– Ig+  |
| Classical                      | CD20–* CD30+ CD15+ Ig– |
| Nodular sclerosis              |                        |
| Mixed cellularity              |                        |
| Lymphocyte rich                |                        |
| Lymphocyte depleted            |                        |

Ig, immunoglobulin.

\*Infrequently positive.

prognosis.<sup>8</sup> A second advance was made in 1966 when Lukes, Butler, and Hicks proposed a classification that related well to clinical presentation and course.<sup>9</sup> Their proposal was slightly modified into the Rye classification, in which four histopathologic subtypes were described: lymphocyte predominant, nodular sclerosis, mixed cellularity, and lymphocyte depleted. In the *World Health Organization Classification of Lymphoid Neoplasms*, the NLPHL subtype is clearly distinguished from cHL.<sup>10</sup> The “lymphocyte-rich” subtype of cHL was introduced in 1999.

Peters described a clinical staging system in 1950, emphasizing the diagnostic evaluation of the anatomic extent of disease.<sup>11</sup> In 1952, Kinmouth introduced lower extremity lymphangiography that allowed roentgenologic visualization of the pelvic and retroperitoneal lymph nodes and was found to be far more sensitive than palpation or other radiographic methods.<sup>12</sup> The frequency of unsuspected splenic involvement was revealed in a group of 65 patients subjected to laparotomy and splenectomy with biopsy of splenic hilar, paraaortic, and mesenteric nodes and liver at Stanford University.<sup>13</sup> These diagnostic procedures led to improved understanding of the mode of dissemination of the disease and correlated well with prognosis, culminating in the modern concepts of staging codified in 1965 at the Rye, New York, conference<sup>14</sup> and further refined at the 1971 Workshop on the Staging of Hodgkin's Disease in Ann Arbor, Michigan.<sup>15</sup>

Pusey (1902)<sup>16</sup> and Senn (1903)<sup>17</sup> were the first to report dramatic regressions of lymphadenopathy with exposure to x-rays, discovered by Roentgen in 1896. Based upon the nearly inevitable recurrence in untreated areas, in 1939 Gilbert proposed the systematic treatment of both involved and uninvolved areas.<sup>18</sup> Peters (1950) is credited for the first demonstration of the curative potential of radiotherapy in her classic paper.<sup>11</sup> The development of megavoltage radiotherapy (doses >4000 cGy), as reported in 1962 by Kaplan,<sup>19</sup> permitted the delivery of tumoricidal doses to virtually all lymphoid regions in the body within acceptable limits of normal tissue tolerance.

The chemotherapy of cHL originated as a byproduct of the war-time work on the mustard gases.<sup>20,21</sup> Following the initial work with the nitrogen mustards, antimetabolites were synthesized, and a number of alkaloids and antibiotics extracted from various plant, fungus, and microbial sources became available for clinical use. DeVita and colleagues introduced the first highly effective combination chemotherapy, MOPP (mechlorethamine [nitrogen mustard], vincristine [Oncovin], procarbazine, and prednisone), based on experimental studies indicating the desirability of combining agents with nonoverlapping toxicities.<sup>22</sup> Combination chemotherapy extended the curative potential for cHL to advanced disease. The ABVD (doxorubicin [Adriamycin], bleomycin, vinblastine, dacarbazine) regimen introduced by Bonadonna and colleagues represented another major advance.<sup>23</sup> Based on a more favorable safety profile and greater efficacy, ABVD replaced MOPP.

## ● EPIDEMIOLOGY

The estimated incidence of Hodgkin lymphoma in the United States was 9190 cases in 2014, with equal incidences in Americans of European and African descent (2.9/100,000).<sup>24</sup> The disease has a median age of onset of 38 years, with bimodal incidence peaks at ages 15 to 34 and older than age 60 years (Fig. 8-1).<sup>25</sup> The second incidence peak is smaller in Americans of European descent and more prominent in Americans of Hispanic descent.<sup>24</sup> Except for Americans of Asian descent, for whom the incidence has increased by 5.2 percent per year, the incidence of Hodgkin lymphoma has been stable in the United States from 1975 to 2011 (Fig. 8-2). The nodular sclerosis subtype predominates in young adults, whereas the mixed cellularity subtype is more common in the pediatric population and at older ages. There is a male predominance at all ages (~1.4:1).

Early studies associated an increased risk of Hodgkin lymphoma in the young adult population with high socioeconomic status.<sup>26</sup> Living in a rental home, sharing a bedroom, and attending daycare or nursery school and early parity in women have been associated with reduced risk. The relationship of incidence to neighborhood socioeconomic status was demonstrated in California for younger but not for older patients.<sup>25</sup> Although associations with occupational exposure to pesticides and lifestyle factors such as cigarette smoking have been reported, the aggregate data do not indicate consistent causal relationships with exogenous chemicals or toxins. A personal or family history of an autoimmune disorder, particularly sarcoidosis, has been associated with an increased risk of Hodgkin lymphoma.<sup>27</sup> Shared etiologic factors with multiple sclerosis have been suggested, but these are thought to be of minor importance.

Geographic patterns vary for the three major age groups: The incidence of Hodgkin lymphoma is greater in childhood in less-developed countries, whereas the incidence peaks in young adulthood and is associated with more favorable histologic subtypes in developed countries.<sup>28</sup> Presence of the Epstein-Barr virus (EBV) in Hodgkin and Reed-Sternberg cells is more common in less-developed countries and in pediatric and older adult cases. The worldwide incidence of the disease is much lower in the Asian population, whether residing in the Far East or in the United States, although the reported rate in Vancouver, Canada, among immigrants of Chinese descent was higher than among Chinese residing in Hong Kong.<sup>29,30</sup> Together, these data suggest a complex interaction

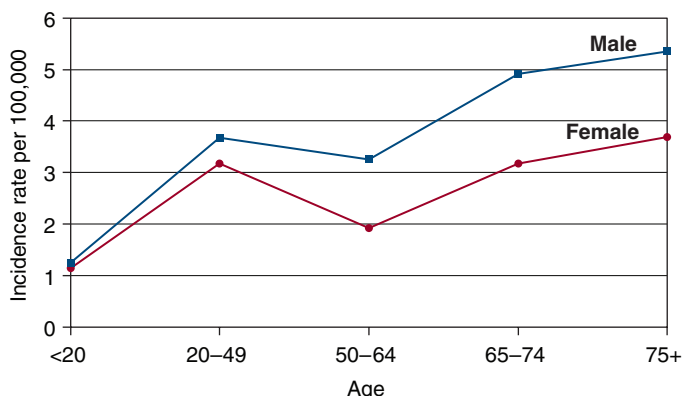


**Figure 8-1.** The graph depicts the incidence of Hodgkin lymphoma as a function of age among American males and females, 2000 to 2011. (Data from the Surveillance, Epidemiology, and End Results [SEER] Program [www.seer.cancer.gov] Research Data (1973–2011), National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2014, based on the November 2013 submission; 2014.)

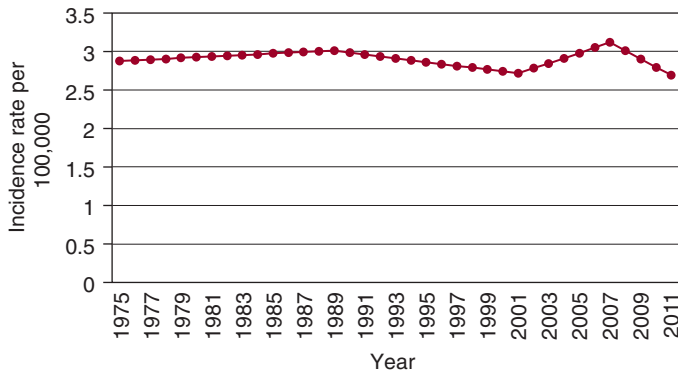


**Figure 8-2.** Incidence of Hodgkin lymphoma by calendar year. (Data from the Surveillance, Epidemiology, and End Results [SEER] Program [www.seer.cancer.gov] Research Data [1973–2011], National Cancer Institute, DCCPS, Surveillance Research Program, Surveillance Systems Branch, released April 2014, based on the November 2013 submission; 2014.)

among possible socioeconomic, environmental, immunologic, genetic, and infectious factors in the incidence of Hodgkin lymphoma.

## ● POSSIBLE INFECTIOUS ETIOLOGY

Demographic features have long supported a “hygiene hypothesis” postulating that one or more subtypes of cHL represent delayed exposure to an infectious agent. In 1966, MacMahon proposed that the first age peak in young adults was infectious in nature, whereas the second peak resulted from causes similar to other lymphomas.<sup>26</sup> As noted, socioeconomic status correlates with the first, but not the second, peak.<sup>25</sup> Several reports of clustering of cHL at the time of diagnosis suggested the possibility of infectious transmission.<sup>31</sup> The weaknesses of the retrospective methodology in these studies have been critically assessed, and further statistical analyses indicated that these likely occurred by chance alone.

A threefold increased risk of cHL in young adults is conferred by a prior history of serologically confirmed infectious mononucleosis. In addition, elevations in titers of EBV, the etiologic agent of infectious mononucleosis, have been reported in patients with cHL.<sup>32,33</sup> A large population study showed that people who developed the disease had abnormally high titers of EBV viral capsid antigen and early antigen in prediagnostic sera.<sup>34</sup> In two subsequent reports, a significantly increased risk of cHL after serologically verified infectious mononucleosis and limited to EBV-positive cases was reported in young adults.<sup>35,36</sup> The median incubation time was approximately 4.1 years.

EBV genomes have been detected in 30 to 50 percent of cHL tissues in developed countries, and EBV-associated cases are more common in cases with mixed cellularity histology, Hispanic ethnicity, and patients older than the age of 60 years.<sup>37–39</sup> Several studies reported a high incidence of EBV association, 85 to 100 percent, in pediatric cHL in which geographic, ethnic, and racial factors have been implicated in the association.<sup>37–39</sup> The incidence of cHL is 10 to 20 times higher in patients with HIV infection than in the general population, and such cases typically have detectable EBV within Hodgkin and Reed-Sternberg cells.<sup>40</sup> In contrast to non-Hodgkin lymphoma, the incidence of cHL in the HIV-infected population has increased despite less-severe immunosuppression in the era of highly active antiretroviral therapy.<sup>41,42</sup>

## ● GENETIC BASIS

Genetic susceptibility and familial aggregation appear to play a role in the incidence of cHL. The increased risk of the disease among identical, but not fraternal, twins provides the strongest evidence for a

genetic association.<sup>43</sup> cHL-prone families, with or without other forms of cancer, have been described in the literature, and it is estimated that 4.5 percent of cases are familial.<sup>44–46</sup> The standard incidence ratio for age-specific familial risk from the Swedish Cancer Registry was higher for Hodgkin lymphoma (4.8) than any other neoplasm.<sup>47</sup> The relative risk for familial disease is stronger in individuals older than 40 years of age, males, and siblings, and a shared risk with chronic lymphocytic leukemia and non-Hodgkin lymphoma has been described.<sup>46</sup> An increased incidence in same-sex siblings (eight- to 12-fold) versus opposite-sex siblings (1.3- to 1.4-fold) detected in the Swedish registry is consistent with older data and has been interpreted to be supportive of an environmental influence or a pseudoautosomal susceptibility gene located on a sex chromosome.<sup>48–50</sup>

Immunoregulatory genes within or near the major histocompatibility complex that may govern susceptibility to viral infections have been postulated to influence susceptibility to cHL, a hypothesis that is supported by the demonstration of lifelong, depressed cellular immunity in cHL patients and their healthy relatives.<sup>51</sup> Several groups described specific human leukocyte antigen (HLA) susceptibility or resistance regions, but these data have been relatively weak and sometimes inconsistent.<sup>52</sup>

## ● ETIOLOGY AND PATHOGENESIS

### ORIGIN OF THE REED-STERNBURG CELL

The histologic diagnosis of cHL is based on the recognition of the Reed-Sternberg cell in an appropriate cellular background. The classic Reed-Sternberg cell has a bilobed nucleus with prominent eosinophilic nucleoli separated by a clear space from the thickened nuclear membrane (Fig. 8-3; see also Chap. 7, Fig. 7-35). Mononuclear variants (Hodgkin cells) have similar nuclear characteristics and may represent Reed-Sternberg cells cut in a plane that shows only one lobe of the nucleus. Reed-Sternberg cells are not pathognomonic for cHL; they may be seen in reactive and other neoplastic conditions. Study of the Reed-Sternberg cell has been complicated by the fact that the neoplastic cells are sparsely interspersed among a reactive, mixed, nonclonal population of lymphocytes, eosinophils, histiocytes, plasma cells, and neutrophils. Difficulty characterizing the neoplastic cells, which account for only 1 to 2 percent of the cellular composition, led to controversy regarding the etiology and pathogenesis of cHL for more than 150 years.

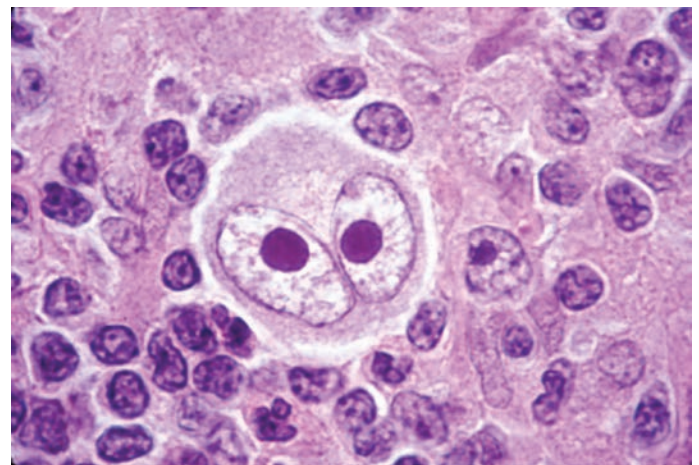


**Figure 8-3.** High magnification of lymph node section in a patient with Hodgkin lymphoma. A Reed-Sternberg cell is in the center of the field with the classical findings of giant size compared to background lymphocytes, binucleation, and prominent eosinophilic nucleoli.

Molecular analyses of single cells obtained by microdissection led to the discovery that cHL and NLPHL are both clonal disorders derived from germinal center B cells, in most cases.<sup>53</sup> The need to survive negative selection in the germinal center, the determination of genetic alterations and constitutive activity of key signaling pathways, and the involvement of EBV in a subset of cases have led to hypotheses concerning the malignant transformation events leading to the formation of Hodgkin/Reed-Sternberg cells. Application of additional genomic technology promises to clarify the molecular changes underlying malignant transformation and cellular proliferation.

### Antigen Receptor Rearrangements

Reed-Sternberg cells and their mononuclear variants demonstrate inconsistent lineage-specific antigen expression that is unlike any other cell of the hematopoietic system. The origin of these cells was eventually determined through isolation of single cells by micromanipulation of histologic sections and analysis for immunoglobulin variable gene rearrangements.<sup>53,54</sup> Nearly all Hodgkin and Reed-Sternberg cells have rearranged and somatically mutated immunoglobulin VH genes, indicating a germinal center or postgerminal center origin of classic Hodgkin and Reed-Sternberg cells.<sup>55–57</sup> Extrapolating from the fact that a subset of these cells carries crippling mutations, it is possible that Hodgkin and Reed-Sternberg cells originate from a preapoptotic germinal center B cell with unfavorable mutations that has escaped negative selection. Rare cases of cHL with a clonal T-cell receptor gene rearrangement have been observed.<sup>58</sup> In contrast, single-cell analyses of NLPHL demonstrated clonal immunoglobulin gene rearrangements with ongoing mutations, an intracлонаl diversity consistent with a germinal center origin of lymphocytic and histiocytic cells.<sup>59–61</sup>

### Reprogramming of Hodgkin and Reed-Sternberg Cells

Hodgkin and Reed-Sternberg cells show a global loss of their B-cell phenotype, retaining only B-cell features associated with their interaction with T cells and their antigen-presenting function.<sup>62</sup> Furthermore, Hodgkin and Reed-Sternberg cells express markers of other lineages, including T cells, dendritic cells, cytotoxic cells, and myeloid cells. The lack of expression of numerous B-cell genes is the result of loss of transcription factor expression (OCT2, BOB1, PU.1) and epigenetic silencing.<sup>63–65</sup> The main B-cell lineage commitment factor, PAX5, is typically expressed, but its target genes are downregulated.<sup>66,67</sup> Reduced expression of target genes likely reflects the fact that B-cell genes are regulated by coordinated action of multiple transcription factors.

The heterogeneity of expression of myeloid, T-cell, dendritic cell, and other genes by Hodgkin and Reed-Sternberg cells is the result of many factors. Early B-cell factor 1 levels are low, de-repressing the expression of T-cell and myeloid genes and lowering transcription of B-cell-specific genes.<sup>68</sup> Notch 1, which plays a key role in promoting T-cell differentiation and inhibiting B-cell development, is expressed in Hodgkin and Reed-Sternberg cells.<sup>69</sup> Notch 1 also contributes to the expression of GATA2, a transcription factor required for proliferation and survival of hematopoietic stem cells.<sup>70</sup> The hematopoietic stem cell regulator polycomb G proteins are also expressed by Hodgkin and Reed-Sternberg cells and are thought to contribute to the expression of markers of different hematopoietic lineages.<sup>71</sup> The signal transducer and activation of transcription factors (STAT) 5A and STAT5B are implicated in Hodgkin and Reed-Sternberg reprogramming as they upregulate CD30 and downregulate B-cell receptor expression.<sup>72</sup> Together, these factors cause a global loss of the B-cell phenotype and aberrant expression of genes of other cell lineages.

### Genetic Alterations and Signaling Pathways

Because Hodgkin and Reed-Sternberg cells lack expression of functional B-cell surface receptors, rescue from apoptosis is probably an important mechanism of survival.<sup>53,73</sup> The most prevalent genetic lesions in Hodgkin and Reed-Sternberg cells involve two signaling pathways: Janus kinase (JAK)-STAT and nuclear factor kappa B (NF- $\kappa$ B). Hodgkin and Reed-Sternberg cells have frequent gains in JAK2 and inactivation of the negative regulator of JAK-STAT signaling, suppressor of cytokine signaling 1, resulting in enhanced cytokine signaling.<sup>74,75</sup> Genetic alterations in NF- $\kappa$ B include gains and amplifications of the NF- $\kappa$ B transcription factor REL in about half the cases of cHL.<sup>76</sup> Somatic mutations of the gene encoding the inhibitor of NF- $\kappa$ B (I $\kappa$ B $\alpha$ ) occur in approximately 20 percent of cases.<sup>77,78</sup> Inactivating mutations and deletions of the gene encoding A20, a negative regulator of NF- $\kappa$ B, have been found in approximately 40 percent of cases, nearly all of which were EBV negative.<sup>79</sup>

Autocrine and paracrine signaling events also contribute to constitutive activation of the JAK-STAT pathway and NF- $\kappa$ B transcription.<sup>72</sup> STAT factors are activated by autocrine means through expression of interleukins (ILs) 13 and 21 and their receptors by Hodgkin and Reed-Sternberg cells and augmented by NF- $\kappa$ B activity.<sup>80–82</sup> Receptor tyrosine kinases expressed in these cells may also contribute to STAT activation. The tumor necrosis factor receptor family, which includes CD30, CD40, transmembrane activator, calcium modulator, and cyclophilin ligand interactor (TACI), B-cell maturation antigen (BCMA), and receptor activator of NF- $\kappa$ B (RANK), is involved in NF- $\kappa$ B signaling through interactions with the cHL microenvironment or in an autocrine fashion.<sup>83,84</sup>

Multiple receptor tyrosine kinases are aberrantly expressed in Hodgkin and Reed-Sternberg cells, including platelet-derived growth factor receptor- $\alpha$ . In addition, deregulated and constitutive activation of the phosphoinositide 3'-kinase (PI3K)-AKT and extracellular signal-regulated kinase (ERK) pathways are implicated in Hodgkin and Reed-Sternberg cells. The activator protein 1 (AP1) transcription factors also appear to play a role, inducing target genes such as galectin 1 and CD30 in Hodgkin and Reed-Sternberg cells.

Several factors point to the pathogenetic role of EBV in approximately 40 percent of cHL. The viral proteins latent membrane protein 1 (LMP1) and latent membrane protein 2 (LMP2), in particular, appear to have hijacked signaling pathways to promote the survival of EBV-infected Hodgkin and Reed-Sternberg cells. LMP1 induces constitutive NF- $\kappa$ B signaling by mimicking the CD40 receptor and can activate JAK-STAT, PI3K, and AP1 signaling. LMP2 functions as a surrogate for the B-cell receptor. The role of EBV in the pathogenesis of cHL also is supported by the findings that (1) there is an inverse relationship between expression of multiple receptor tyrosine kinases and EBV expression, (2) there is an ability of EBV to rescue crippled germinal center B cells in the laboratory, (3) mutations preventing any B-cell receptor expression are in EBV-positive Hodgkin and Reed-Sternberg cells, and (4) there is an inverse relationship between mutations reducing the expression of the NF- $\kappa$ B regulator A20 and EBV-positive Hodgkin and Reed-Sternberg cells.

Overall, genetic alterations involving the JAK-STAT and NF- $\kappa$ B signaling pathways and further activation via autocrine or paracrine mechanisms interact to support the growth and survival of cHL cells. In the EBV-positive subset of patients, viral genes can provide the pathogenetic function of genetic lesions found in EBV-negative cases.

### ROLE OF THE MICROENVIRONMENT

The survival of Hodgkin and Reed-Sternberg cells appears to be dependent on their microenvironment, which represents 95 to 99 percent of the cellular composition of the tumor. Hodgkin and Reed-Sternberg



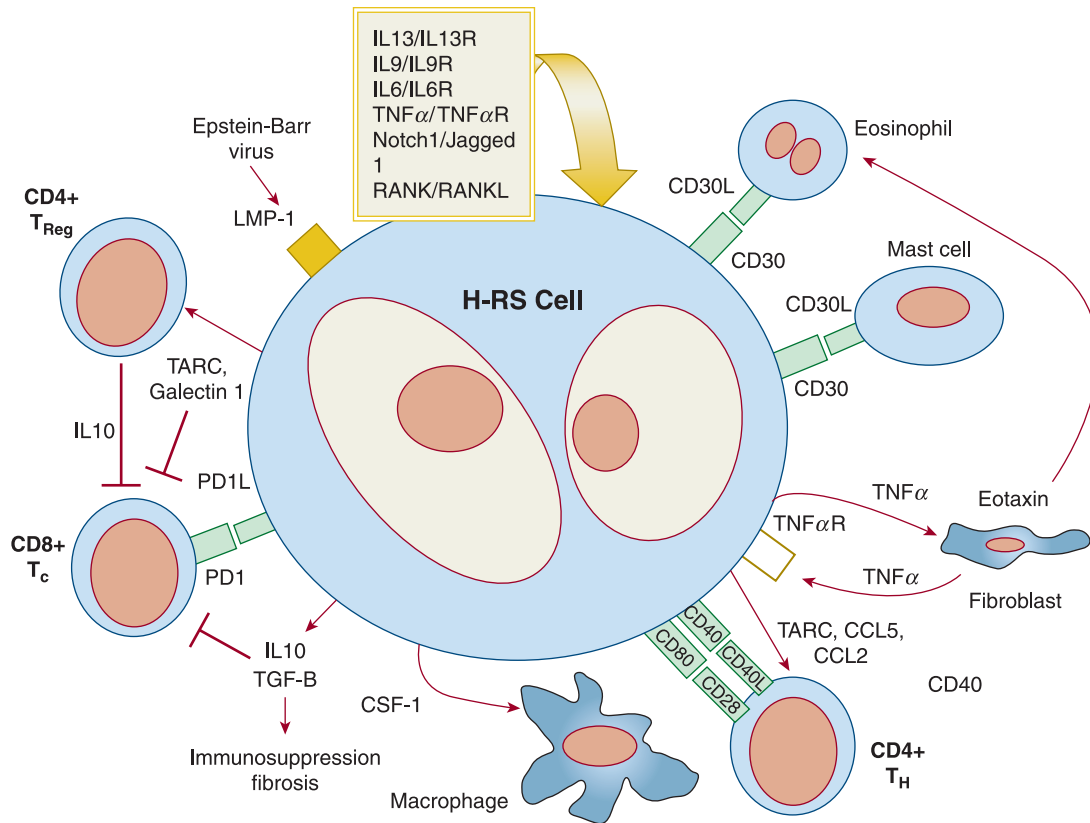


**Figure 8-4.** The Reed-Sternberg cell and its environment. In a network of highly complex interactions, Reed-Sternberg cells elaborate chemokines that attract a variety of cells, which cascade their influence on the microenvironment. Reed-Sternberg cells express ligands that play a role in auto-crine and paracrine interactions and also produce a number of immunosuppressive factors (e.g., galectin 1) that directly contribute to a protumor, humoral T-helper 2 environment (the “Role of the Microenvironment” section provides further details). CSF, colony-stimulating factor; H-RS, Hodgkin/Reed-Sternberg; IL, interleukin; LMP, latent membrane protein; PD1, programmed cell death protein 1; RANK, receptor activator of nuclear factor- $\kappa$ B; TARC, thymus and activation-related chemokine; TGF- $\beta$ , transforming growth factor beta; TNF, tumor necrosis factor.

cells attract T cells, B cells, neutrophils, plasma cells, eosinophils and mast cells by secreting chemokines (Fig. 8-4). For instance, CCL5, CCL17, and CCL22 attract T-helper 2 and T-regulatory cells. Other chemokines attract eosinophils and mast cells, and IL-8 attracts neutrophils. These chemokines may also have direct effects on Hodgkin and Reed-Sternberg cells. T cells represent the largest and probably most important population. CD4<sup>+</sup> T cells trigger CD40 signaling, and CD4<sup>+</sup> T-regulatory cells have potent immunosuppressive activity against infiltrating cytotoxic T cells. Other interactions include activation of TACI and BCMA through their ligand production by neutrophils and activation of CD30 through CD30 ligand-expressing mast cells and eosinophils. Connective tissue cells and their products can be involved in complex interactions, such as the stimulation of fibroblasts via factors expressed by Hodgkin and Reed-Sternberg cells and the consequent secretion by these fibroblasts of eotaxin and CCL5, which attract eosinophils and T-regulatory cells to the cHL microenvironment.

The differentiation of CD4<sup>+</sup> T cells to T-regulatory cells and the immunosuppressive features of the cHL microenvironment have received much attention. A hallmark of these changes is the shift from an antitumor, cytotoxic T-helper 1 response to a protumor, humoral T-helper 2 response. Hodgkin and Reed-Sternberg cells produce a number of immunosuppressive factors, such as IL-10, transforming growth factor- $\beta$ , galectin 1, and prostaglandin  $E_2$ . Hodgkin and Reed-Sternberg cells express programmed cell death protein 1 (PD-1) ligand, which binds and inhibits T-cell cytotoxic function.

## CLINICAL FEATURES

### PRESENTING MANIFESTATIONS

#### History and Physical Examination

Constitutional symptoms accompany the diagnosis of cHL in approximately 30 percent of cases. Fever in excess of 38°C, drenching night sweats, and weight loss exceeding 10 percent of baseline body weight during the 6 months preceding diagnosis are designated as symptomatic “B” disease. Fevers are usually of low grade and irregular. Rarely, a cyclic pattern of high fevers for 1 to 2 weeks alternating with afebrile periods of similar duration, known as Pel-Ebstein fever, is present at diagnosis and is virtually diagnostic of the disease.<sup>85,86</sup> Generalized pruritus, often accompanied by marked excoriation, may be present at diagnosis but does not confer prognostic significance. Pain in involved lymph nodes immediately after the ingestion of alcohol is a curious complaint that occurs in fewer than 10 percent of patients but is nearly specific to cHL.<sup>87</sup> The etiology of these symptoms has been the subject of speculation but remains largely unexplained. Patients with extensive intrathoracic disease may present with cough, chest pain, dyspnea, and, rarely, hemoptysis. Infrequently, patients present with bone pain, including the constellation of back pain accompanied by signs and symptoms of spinal cord compression.

Detection of an unusual mass or swelling in the superficial, supradiaphragmatic lymph nodes (60 to 70 percent cervical and supraclavicular,

15 to 20 percent axillary) is the most common presentation of cHL. Only 15 to 20 percent of patients have subdiaphragmatic disease at presentation.<sup>88</sup> Lymphadenopathy is usually nontender and has a “rubbery” consistency. By inspection, a diffuse, puffy swelling rather than a discrete mass may be apparent in the supraclavicular, infraclavicular, or anterior chest wall regions. Infrequently, compression of the superior vena cava will result in facial swelling and engorgement of the veins in the neck and upper chest. Auscultation of the chest may reveal a pleural effusion. Rarely, a significant pericardial effusion is present at diagnosis. Palpation of the abdomen may reveal intraabdominal masses or hepatosplenomegaly, although physical examination is relatively insensitive for detection of these abnormalities.

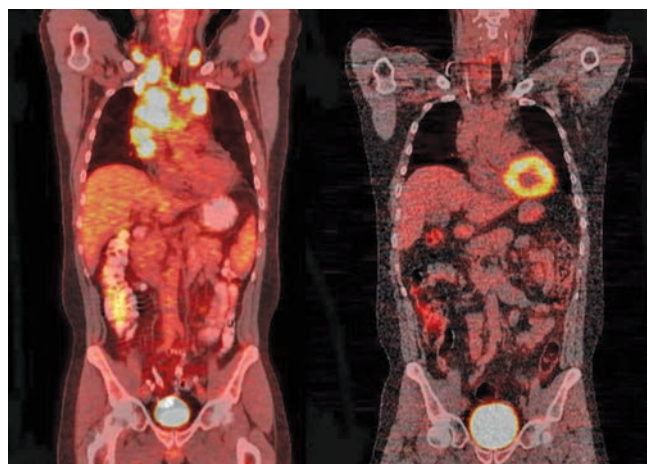
### Paraneoplastic Findings

A number of rare paraneoplastic syndromes have been described in cHL at the time of diagnosis. These include “vanishing bile duct syndrome” and idiopathic cholangitis with clinical jaundice, the nephrotic syndrome with anasarca, autoimmune hematologic disorders (e.g., immune thrombocytopenia or hemolytic anemia), and neurologic signs and symptoms.<sup>89–91</sup> Although parenchymal involvement of the central nervous system or meningeal involvement is rare in cHL, paraneoplastic syndromes include subacute cerebellar degeneration, myelopathy, progressive multifocal encephalopathy, and limbic encephalitis.<sup>91,92</sup>

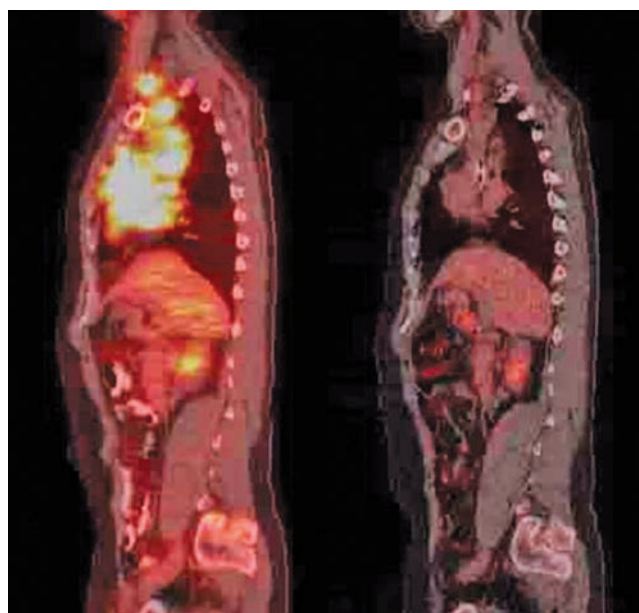
## RADIOGRAPHIC FEATURES

Intrathoracic disease is present at diagnosis in two-thirds of patients. Mediastinal adenopathy is common in cHL, particularly in young women with the nodular sclerosis subtype.<sup>93</sup> Hilar adenopathy, pulmonary parenchymal involvement, pleural effusions, pericardial effusions, and chest wall masses may be appreciated by chest computed tomography (CT); these are more common in the presence of extensive mediastinal disease. CT of the abdomen and pelvis is routinely employed in the diagnostic evaluation of cHL. Although technologic advances have greatly increased the resolution of this technique and the subsequent detection of celiac, portal, splenic hilar, and mesenteric lymph nodes, the correlation with histologic involvement of the spleen, historically determined by laparotomy staging, has been disappointing.

Whole-body <sup>18</sup>F-fluorodeoxyglucose positron emission tomography (FDG-PET) has become standard in the staging of cHL (Fig. 8–5).<sup>94,95</sup> FDG-PET correlates well with CT evaluation and may demonstrate additional areas of disease, although this information uncommonly results in changes in stage or choice of initial therapy.<sup>95,96</sup> FDG-PET, however, is more sensitive to bone and hepatic disease, and a diffuse increase in signal can be seen at diagnosis in patients with neutrophilia. Additionally, FDG-PET is sensitive for the presence of marrow involvement at diagnosis, and the absence of marrow uptake obviates the need for marrow biopsy.<sup>96a</sup> FDG-PET imaging is superior to CT scanning in distinguishing active residual disease (increased glucose metabolism) from inactive residual tissue, a major problem in assessing remission status after treatment, and has been incorporated in formal revised response guidelines.<sup>94,95</sup> False-positive FDG-PET scans can be seen in the marrow during or at the end of treatment as a consequence of the chemotherapy effect or use of hematopoietic colony-stimulating factors. In followup, false-positive studies may be caused by thymic hyperplasia, granulomatous disease, or infectious disorders. In addition to evaluation of residual masses, FDG-PET has been incorporated in early response monitoring for risk stratification and, in clinical trials, to alter therapy.<sup>96–102</sup> The predictive accuracy of FDG-PET is dependent on expertise of the imaging staff and clinical correlation. In most situations, but particularly in FDG-PET, avid anatomic sites that were previously uninvolved or those without concomitant abnormality on



**A**



**B**

**Figure 8–5.** <sup>18</sup>Fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) imaging of classical Hodgkin lymphoma. **A.** Coronal views of whole-body FDG-PET/CT imaging performed before and after therapy for stage IIB classical Hodgkin lymphoma with Adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) chemotherapy and involved-field radiotherapy. At the time of diagnosis, hypermetabolic areas of avid-FDG uptake were evident in bilateral cervical, supraclavicular, mediastinal, and hilar lymph nodes. Physiologic FDG uptake was visible in the colon and bladder. After completion of therapy, there was no abnormal hypermetabolic activity in any of the original sites of disease, although physiologic FDG activity was seen in the cardiac blood pool. **B.** Sagittal views of the same patient before and after therapy, showing resolution of hypermetabolism in all involved nodal sites. The patient has remained in complete remission since finishing therapy.

CT scan usually require tissue biopsy confirmation. Combined CT and FDG-PET technology is now standard for staging and posttreatment response evaluation and has resulted in improved anatomic definition of sites with increased signal.<sup>94–96</sup>

## ● CLINICAL AND PATHOLOGIC CORRELATION

### NODULAR LYMPHOCYTE PREDOMINANCE SUBTYPE

There is a strong correlation between age at onset, the anatomic extent of disease, and histologic subtype of Hodgkin lymphoma. Approximately 5 to 10 percent of patients present with NLPHL, which is considered to be a distinct subtype, discrete from cHL.<sup>103,104</sup> Progressive transformation of germinal centers may precede or follow NLPHL in other sites. The cellular composition is predominantly benign B lymphocytes with or without histiocytes. The characteristic multilobated, CD20+ lymphocytic and histiocytic cells are relatively abundant (see Chap. 7, Fig. 7–40). Patients most commonly present with stage I disease (70 percent) in peripheral lymph node sites, particularly in the axillae, and there is a 3 to 4:1 male predominance.<sup>103,104</sup> NLPHL may be associated with diffuse large B-cell non-Hodgkin lymphoma as a composite tumor or a large cell lymphoma may develop at a later date.<sup>105,106</sup> The large cell variant, T-cell-rich B-cell lymphoma, may be difficult to distinguish from NLPHL and may occur concurrently or subsequently (Chap. 9).<sup>107</sup>

### NODULAR SCLEROSIS SUBTYPE

cHL is characterized by Hodgkin and Reed-Sternberg cells that express CD30 and CD15+ but usually not typical B-cell surface markers such as CD20 or CD79B. The nodular sclerosis subtype constitutes 40 to 70 percent of cHL and is distinguished by its distinctive clinical and histologic features. This subtype typically involves lower cervical, supraclavicular, and mediastinal lymph nodes in adolescents and young adults, particularly females. Approximately 70 percent of patients with the nodular sclerosis subtype present with limited stage disease. A characteristic histologic feature is the lacunar cell, a Reed-Sternberg variant that results from retraction of the cytoplasm of Hodgkin and Reed-Sternberg cells during formalin fixation (see Chap. 7, Fig. 7–38). Another typical feature is the thickened capsule and fibrous bands that divide the lymphoid tissue into cellular nodules.

### MIXED CELLULARITY SUBTYPE

Mixed cellularity cHL involves both pediatric and older age groups and is more commonly associated with advanced-stage disease, constitutional symptoms, and immunodeficiency. Approximately 30 to 50 percent of patients present with this histology. Classic Hodgkin and Reed-Sternberg cells are easily found amid a cellular background composed of lymphocytes, eosinophils, plasma cells, and histiocytes (see Chap. 7, Fig. 7–39). A worse prognosis has been reported for this subtype.<sup>108</sup>

### LYMPHOCYTE-DEPLETED SUBTYPE

Lymphocyte-depleted cHL has two morphologic variants: reticular and diffuse fibrosis. The reticular variant contains abundant pleomorphic neoplastic cells, whereas the diffuse fibrosis variant has a prominent fibroblastic proliferation with few normal lymphocytes. Hodgkin and Reed-Sternberg cells are sparse. The lymphocyte-depleted subtype presents in older patients with symptomatic, extensive disease and may be associated with fever of unknown origin, jaundice, hepatosplenomegaly, or pancytopenia. Peripheral and mediastinal adenopathy is much less common than in other subtypes. Cases of cHL developing in the setting of HIV typically exhibit the lymphocyte-depleted morphology.

### LYMPHOCYTE-RICH SUBTYPE

The lymphocyte-rich subtype of cHL was introduced by the World Health Organization classification in 1999 (see Table 8–1) following an expert pathology review of cases of NLPHL.<sup>109</sup> The two subtypes differ subtly on morphologic grounds, but the major difference is that the Hodgkin and Reed-Sternberg cells in lymphocyte-rich cHL exhibit the classic CD30+ CD20– immunophenotype. The presenting features are very similar, although patients with the lymphocyte-rich subtype tend to be older compared with NLPHL patients.<sup>109</sup> A higher rate of multiple relapses and a more favorable prognosis upon relapse is characteristic of NLPHL.

## ● ANATOMIC DISTRIBUTION OF DISEASE

In approximately 70 percent of patients, cHL presents in the cervical nodes; in 12 percent, in the axillary nodes; and in 9 percent, in the inguinal nodes.<sup>110</sup> A small minority of patients presents exclusively with subdiaphragmatic disease. In a historical series of 285 consecutive, unselected, and untreated patients evaluated at Stanford University, involvement of abdominal lymph nodes and the spleen was documented in 272 upon laparotomy, a surgical diagnostic procedure in which the intraabdominal and pelvic lymph nodes are biopsied, the spleen is removed and examined pathologically in thin slices, the liver is biopsied by needle and wedge technique, and the marrow is biopsied. The frequency of splenic involvement at laparotomy in untreated patients averaged 37 percent in 17 published series.<sup>110</sup> Involvement of the spleen was strongly dependent on histologic subtype: It was involved in 60 percent of mixed cellularity and lymphocyte-depleted cases compared with 34 percent of nodular lymphocyte-predominant and nodular sclerosis cases. Hepatic and marrow disease were invariably associated with splenic involvement.

Two different theories, the “contiguity” theory of Kaplan and Rosenberg<sup>111</sup> and the “susceptibility” theory of Smithers,<sup>112</sup> have been proposed for the mode of spread of cHL. In support of the former, most cases of cHL appear to spread via lymphatic channels to contiguous lymphatic structures in a predictable, nonrandom pattern. Controversy has surrounded the mode of spread to the spleen, which lacks afferent lymphatics. When four or more lymph node regions are involved, spread by hematogenous distribution appears likely. Disseminated disease is more common in mixed cellularity and lymphocyte-depleted cases, consistent with the presence of reported vascular invasion.<sup>113</sup> Whereas vascular invasion is controversial, it is more common in the spleen than in lymph nodes and connotes a poor prognosis.<sup>114</sup>

## ● STAGING

Optimal management of Hodgkin lymphoma relies on knowledge of the extent of disease dissemination, which is determined by performing a careful physical examination, supplemented by laboratory tests and radiographic imaging studies (Chap. 6). This process, known as “staging,” has been refined by a series of international working groups. The current 2014 Lugano staging system<sup>94</sup> is a refinement of the historical Ann Arbor classification<sup>15</sup> and assigns patients to one of four stages as indicated in Table 8–2. The classification for Hodgkin lymphoma is further refined by designating the presence or absence of constitutional B symptoms (fever >38°C, weight loss >10 percent of body weight, or drenching night sweats). Extranodal disease, representing extracapsular extension of lymph node disease that could be incorporated in a standard radiotherapy field, is distinguished from disseminated, stage IV disease. The correlation of this staging classification system with prognosis was extensively verified when



**TABLE 8-2. Staging System for Hodgkin Lymphoma****Stage**

- I. Involvement of a single lymph node region (I) or a single extralymphatic organ or site (I<sub>E</sub>)
- II. Involvement of two or more lymph node regions on the same side of the diaphragm alone (II) or with involvement of limited, contiguous extralymphatic organ or tissue (II<sub>E</sub>)
- III. Involvement of lymph node regions on both sides of the diaphragm (III), which may include the spleen (III<sub>S</sub>) or limited, contiguous extralymphatic organ or site (III<sub>E</sub>) or both (III<sub>ES</sub>)
- IV. Multiple or disseminated foci of involvement of one or more extralymphatic organs or tissues, with or without associated lymph node involvement

**Modifying Features**

- A. Asymptomatic
- B. Drenching night sweats; fever >38°C; loss of more than 10% body weight in 6 months
- C. Involvement of a single, contiguous or proximal extranodal site

Bulky disease is defined as a mass >10 cm or a mediastinal mass ratio >0.33. The mediastinal mass ratio is the ratio of the maximal width of a mediastinal mass relative to the maximal width of the mediastinum, as measured by computed tomographic imaging.

radiotherapy served as the principal treatment for all but stage IV disease. Recommended staging procedures for untreated patients have evolved with changes in therapy. Exploratory laparotomy and splenectomy, which historically advanced about one-third of clinical stages I and II patients to pathologic stages III and IV, is no longer performed. CT of the chest, abdomen, and pelvis and FDG-PET imaging provide sensitive delineation of involved sites, and the use of chemotherapy in all stages of disease has reduced the critical nature of detecting subclinical disease. Marrow involvement occurs in approximately 12 percent of new patients and is more common in patients of older age, advanced stage, less-favorable histology, or those with constitutional symptoms or immunodeficiency. Because the marrow is almost never involved in young, asymptomatic patients with favorable clinical stage I or II presentations, marrow biopsy may be omitted in staging such patients, particularly if PET-CT is negative.

## LABORATORY FEATURES

There are no diagnostic laboratory features of cHL. A complete blood count may reveal granulocytosis, eosinophilia, anemia, lymphocytopenia, or thrombocytosis. Anemia is usually the result of “chronic disease,” but rarely may be caused by hemolysis associated with a positive direct antiglobulin (Coombs) test. Thrombocytopenia may occur as a result of marrow involvement, hypersplenism, or an immune mechanism. Immune neutropenia can occur in cHL. Cytopenias are particularly common in advanced-stage disease and the lymphocyte-depleted subtype. Elevation of the erythrocyte sedimentation rate (ESR) is most common in advanced disease and correlates with constitutional symptoms.<sup>115,116</sup> The degree of sedimentation rate elevation correlates with prognosis, particularly in limited-stage disease.<sup>117</sup> Although nonspecific, it may be a useful harbinger of recurrent disease if serially monitored. Serum lactate dehydrogenase levels are elevated in 35 percent of patients at diagnosis.<sup>118,119</sup> The alkaline phosphatase may be elevated in cHL, nonspecifically in limited disease, or in association with involvement of liver, bone, or marrow in advanced disease. Hypercalcemia is unusual in cHL and when present may be secondary to synthesis of

increased levels of 1,25-dihydroxyvitamin D by Hodgkin lymphoma cells.<sup>120</sup> A variety of other abnormalities have been reported, including hypoglycemia<sup>121,122</sup> resulting from autoantibodies to insulin receptors and hyponatremia resulting from inappropriate secretion of antidiuretic hormone.<sup>123</sup>

Anemia, granulocytosis, lymphopenia, and low serum albumin constitute four of seven adverse prognostic factors identified in advanced Hodgkin lymphoma by an international consortium.<sup>124</sup> Similar to the non-Hodgkin lymphomas, serum  $\beta_2$ -microglobulin levels correlate with tumor burden and prognosis in Hodgkin lymphoma.<sup>125</sup> Serum levels of cytokines, including soluble CD30, IL-6, IL-10, and the IL-2 receptor, have been reported to correlate with constitutional symptoms and advanced disease.<sup>126–129</sup> Examination of pleural fluid in Hodgkin lymphoma may reveal transudative, exudative, or chylous characteristics. Because cytology rarely yields diagnostic Hodgkin and Reed-Sternberg cells, the etiology is most often considered to be one of central lymphatic obstruction. Laboratory abnormalities, including abnormal liver function tests associated with marked enlargement of porta hepatis nodes and biliary obstruction or intrahepatic cholestasis, may be prominent in rare presentations of Hodgkin lymphoma.<sup>130</sup> Similarly, the nephrotic syndrome, characterized by edema, azotemia, hypoalbuminemia, and hyperlipidemia, may rarely be present at the time of diagnosis of Hodgkin lymphoma.<sup>131</sup>

## DIFFERENTIAL DIAGNOSIS

Clinically enlarged lymph nodes may be associated with a variety of infectious, inflammatory, autoimmune, and neoplastic disorders. Biopsy of unexplained, persistent, or recurrent adenopathy should be reviewed by an experienced hematopathologist. Distinction of cHL<sup>132</sup> from primary mediastinal B-cell lymphoma may be difficult based on both clinical and histologic features. Gene expression profiling studies suggest that these disorders are closely related pathogenetically.<sup>132–135</sup> Mixed cellularity Hodgkin lymphoma may demonstrate varied cellular and stromal compositions and must be distinguished from peripheral T-cell lymphoma and T-cell-rich B-cell lymphoma, which can also be difficult to distinguish from NLPHL.<sup>136</sup> Immune markers of Hodgkin and Reed-Sternberg cells, such as CD30, CD20, and CD15, are invaluable for differential diagnosis (see Chap. 7, Figs. 7–36 and 7–41). Non-neoplastic conditions that simulate Hodgkin lymphoma include viral infections, particularly infectious mononucleosis. Depleted nodes of any histology, including the depleted phase of lymph nodes from HIV-infected patients, may resemble the diffuse fibrosis variant of lymphocyte-depleted Hodgkin lymphoma. Histologic assessment of extranodal sites depends upon the organ involved and whether there is a known diagnosis of cHL. Identification of typical Hodgkin and Reed-Sternberg cells in needle biopsies of liver and marrow biopsies is not required because the specimens for evaluation are typically very small.

## THERAPY

### HISTORICAL PERSPECTIVE

Hodgkin lymphoma first became a curable neoplasm through the systematic study of the spread of the disease and the use of higher dose, extended-field radiotherapy delivered with supervoltage techniques.<sup>137</sup> When used alone, radiotherapy doses to involved fields usually ranged from 3500 to 4400 cGy with prophylactic doses of 3000 to 3500 cGy to uninvolved tissues. The mantle, paraaortic region, and pelvis constitute the classic radiotherapy regions. With increased recognition of late effects, radiation therapy has been modified to reduce field size to areas of known or bulky disease and doses have been lowered, coincident

with addition of systemic chemotherapy. Furthermore, initial disease reduction with chemotherapy results in less radiation exposure to the neck, female breast, heart, and lungs, all of which are anticipated to result in fewer late complications. Advances in radiotherapy techniques deliver more precise dose distributions, sparing normal tissues. The first modern combination chemotherapy program was the MOPP regimen devised by Devita and colleagues.<sup>22</sup> The national mortality figures for cHL decreased by more than 60 percent in the decade that followed the introduction of MOPP.<sup>138</sup> Bonadonna and colleagues developed an important alternative regimen for the treatment of cHL. ABVD, which was effective in the treatment of patients who had failed MOPP,<sup>139,140</sup> offered a more favorable toxicity profile. ABVD subsequently became the preferred primary chemotherapy regimen, alone or in combination with radiotherapy.<sup>141,142</sup> Of the multiple alternative chemotherapy regimens introduced for the treatment of advanced cHL, only the bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, procarbazine (BEACOPP) combination developed by Diehl and colleagues has demonstrated superior cure rates in multiple phase III studies.<sup>143,144</sup> Table 8–3 describes the drugs, doses, and schedules of combination chemotherapy programs effective in the management of Hodgkin lymphoma.

### FAVORABLE, LIMITED-STAGE DISEASE

Favorable, limited-stage Hodgkin lymphoma is typically defined in North America as asymptomatic stage I or II supradiaphragmatic disease with no bulky sites. A more restrictive definition is used in Europe based on the number of Ann Arbor sites, ESR, age, and extranodal sites, as well as bulky disease (Table 8–4).<sup>143</sup> Approximately 35 percent of stages I and II patients meet this more limited definition of favorable disease. For many years, extended-field (subtotal lymphoid) radiotherapy administered after staging laparotomy was the treatment of choice for early stage, favorable Hodgkin lymphoma. A change in that standard was compelled by the observation that the overall mortality rate from other causes, particularly second cancers, exceeded deaths resulting from Hodgkin lymphoma at 15 to 20 years.<sup>145</sup> Early studies from Stanford University demonstrated that involved-field radiotherapy plus chemotherapy produced results equivalent or superior to wide-field radiotherapy.<sup>146</sup> Subsequently, several randomized trials demonstrated the superiority of involved-field radiotherapy plus anthracycline-containing chemotherapy compared to extended-field radiotherapy in early stage favorable Hodgkin lymphoma.<sup>147,148</sup>

The next series of clinical trials were designed to test the optimal number of cycles of chemotherapy and the volume and dose of radiotherapy when both modalities are used in limited Hodgkin lymphoma. The Milan Tumor Institute documented disease control in more than 95 percent of early stage cHL patients treated with four cycles of ABVD and radiotherapy, with no advantage seen for extended-field radiotherapy compared to involved-field radiotherapy.<sup>149</sup> Similarly, no advantage to more extensive radiation in combination with chemotherapy was observed in a German Hodgkin Study Group (GHSG) study.<sup>150</sup> A comparison of two versus four cycles of ABVD chemotherapy paired with 20- or 30-Gy radiotherapy was made in a four-arm trial conducted by the GHSG, evaluating patients with “favorable” early stage cHL, defined as patients with two or fewer sites of disease, no masses larger than 10 cm, a normal ESR, and no extranodal sites of disease. This trial produced equivalent outstanding outcomes for all four arms, with 91 to 93 percent freedom from treatment failure after 5 years, and established two cycles of ABVD followed by 20 Gy of involved-field radiotherapy as a new standard of care for early stage, favorable cHL. In a subsequent study, the GHSG evaluated eliminating drugs from the ABVD regimen, randomizing patients between four cycles of ABVD, AV, ABV, or AVD plus 30-Gy

involved-field radiotherapy. The AV and ABV arms were closed early because of clearly inferior outcomes, and subsequent analysis suggested that the AVD arm is also worse than the full four-drug combination.<sup>151</sup>

The high cure rate with current limited chemotherapy and low-dose radiotherapy creates a high standard for comparison with alternative treatment approaches. Nevertheless, considerable interest exists in devising management strategies omitting radiotherapy altogether, largely motivated by desires to avoid secondary malignancies and late cardiopulmonary complications.<sup>145</sup> This approach is particularly favored for women younger than age 30 years, who have a very high risk of developing breast cancer if treated with mediastinal radiotherapy.<sup>145,152</sup> Canellos and colleagues treated 71 patients with early stage, favorable cHL with six cycles of ABVD without radiotherapy and achieved a 5-year failure-free survival of 92 percent.<sup>153</sup> A single-institution study of ABVD versus ABVD plus radiotherapy demonstrated no significant progression-free survival difference between the treatment arms, but this trial accrued relatively small numbers of patients.<sup>154</sup> A phase III North American study assigned 405 patients with previously untreated stage IA or IIA nonbulky Hodgkin lymphoma to treatment with ABVD alone for four to six cycles or subtotal nodal radiation therapy, with or without ABVD. Among those assigned to subtotal nodal radiation therapy, patients who had a favorable risk profile received subtotal nodal radiation therapy alone, and patients with an unfavorable risk profile received two cycles of ABVD plus subtotal nodal radiation therapy. After a median followup of 11.3 years, the overall survival (OS) was 94 percent among those receiving ABVD alone, compared to 87 percent for those receiving subtotal nodal radiation therapy ( $p = 0.04$ ). The rates of freedom from disease progression were 87 percent and 92 percent in the two groups, respectively ( $p = 0.05$ ). The investigators concluded that treatment with ABVD therapy alone was superior because of a lower rate of death from secondary malignancies and other causes,<sup>155</sup> although critics have emphasized that the large fields and high doses of radiotherapy used in this trial are obsolete and that modern radiotherapy techniques would be anticipated to have much more favorable outcomes. In a European trial, the epirubicin, bleomycin, vinblastine, prednisone (EBVP) regimen was tested against the same chemotherapy plus 20- or 30-Gy involved-field radiotherapy.<sup>156</sup> Inferiority of the EBVP combination without radiotherapy resulted in the trial's early closure. A Cochrane meta-analysis of randomized controlled trials comparing chemotherapy alone with combined modality therapy concluded that adding radiotherapy to chemotherapy improves tumor control and OS in patients with early stage Hodgkin lymphoma.<sup>157</sup>

One focus of current studies is to assess the potential role of interim FDG-PET scanning as a means of identifying patients (PET negative) for whom radiotherapy can be omitted. The European Organization for Research and Treatment of Cancer (EORTC), the Lymphoma Study Association (LySA), and Fondazione Italiana Linfomi (FIL) H10 trial randomized 1137 patients with untreated supradiaphragmatic clinical stage I/II cHL to either standard therapy or an experimental, PET-response-adapted approach. Patients in the trial were stratified into favorable and unfavorable cohorts based on the presence or absence of adverse risk factors (age  $\geq 50$  years, more than four involved nodal areas, presence of mediastinal bulk [mediastinum-to-thorax ratio  $\geq 0.35$ ], or ESR  $\geq 50$  mm without B symptoms or ESR  $\geq 30$  mm with B symptoms).<sup>101</sup> The favorable group was randomized to either standard therapy with three cycles of ABVD chemotherapy followed by involved nodal radiotherapy (30 Gy + 6-Gy boost) or “experimental therapy” in which patients whose interim PET scans after two cycles of ABVD were negative received two more cycles of ABVD (total of four cycles) but no radiotherapy. Patients in the experimental arm who had FDG-avid foci of disease after two cycles of ABVD were switched to the BEACOPP-escalated regimen for two cycles followed by involved nodal radiotherapy.

**TABLE 8-3.** Combination Chemotherapy for Hodgkin Lymphoma

| Drug                                                             | Dose (mg/m <sup>2</sup> ) | Route | Schedule (Days Administered)   | Cycle Length (Days) |
|------------------------------------------------------------------|---------------------------|-------|--------------------------------|---------------------|
| ABVD                                                             |                           |       |                                | 28                  |
| Doxorubicin                                                      | 25                        | IV    | 1, 15                          |                     |
| Bleomycin                                                        | 10                        | IV    | 1, 15                          |                     |
| Vinblastine                                                      | 6                         | IV    | 1, 15                          |                     |
| Dacarbazine                                                      | 375                       | IV    | 1, 15                          |                     |
| COPP                                                             |                           |       |                                | 28                  |
| Cyclophosphamide                                                 | 650                       | IV    | 1, 8                           |                     |
| Vincristine                                                      | 1.4*                      | IV    | 1, 8                           |                     |
| Procarbazine                                                     | 100                       | PO    | 1–14                           |                     |
| Prednisone                                                       | 40                        | PO    | 1–14                           |                     |
| COPP/ABVD                                                        |                           |       |                                | 28                  |
| Alternate cycles of COPP with ABVD                               |                           |       |                                |                     |
| BEACOPP (standard)                                               |                           |       |                                | 21                  |
| Bleomycin                                                        | 10                        | IV    | 8                              |                     |
| Etoposide                                                        | 100                       | IV    | 1–3                            |                     |
| Doxorubicin                                                      | 25                        | IV    | 1                              |                     |
| Cyclophosphamide                                                 | 650                       | IV    | 1                              |                     |
| Vincristine                                                      | 1.4*                      | IV    | 8                              |                     |
| Procarbazine                                                     | 100                       | PO    | 1–7                            |                     |
| Prednisone                                                       | 40                        | PO    | 1–14                           |                     |
| BEACOPP (escalated)                                              |                           |       |                                | 21                  |
| Bleomycin                                                        | 10                        | IV    | 8                              |                     |
| Etoposide                                                        | 200                       | IV    | 1–3                            |                     |
| Doxorubicin                                                      | 35                        | IV    | 1                              |                     |
| Cyclophosphamide                                                 | 1250                      | IV    | 1                              |                     |
| Vincristine                                                      | 1.4*                      | IV    | 8                              |                     |
| Procarbazine                                                     | 100                       | PO    | 1–7                            |                     |
| Prednisone                                                       | 40                        | PO    | 1–14                           |                     |
| G-CSF                                                            | (+)                       | SQ    | 8+                             |                     |
| BEACOPP (14-day)                                                 |                           |       |                                | 14                  |
| Standard BEACOPP given every 14 days with growth factor support. |                           |       |                                |                     |
| STANFORD V                                                       |                           |       |                                | 12 weeks            |
| Nitrogen mustard                                                 | 6                         | IV    | Day 1 on wk 1, 5, 9            |                     |
| Doxorubicin                                                      | 25                        | IV    | Day 1 on wk 1, 3, 5, 7, 9, 11  |                     |
| Vinblastine                                                      | 6                         | IV    | Day 1 on wk 1, 3, 5, 7, 9, 11  |                     |
| Vincristine                                                      | 1.4*                      | IV    | Day 1 on wk 2, 4, 6, 8, 10, 12 |                     |
| Bleomycin                                                        | 5                         | IV    | Day 1 on wk 2, 4, 6, 8, 10, 12 |                     |
| Etoposide                                                        | 60×2                      | IV    | Day 1 & 2 on wk 3, 7, 11       |                     |
| Prednisone                                                       | 40                        | PO    | Day 1 on wk 1–10, taper        |                     |
| G-CSF for dose reduction, delay                                  |                           |       |                                |                     |

G-CSF, granulocyte colony-stimulating factor; SQ, subcutaneously. See text for definitions of the combination treatment programs.

\*Capped at 2 mg.



**TABLE 8–4.** Prognostic Factors for Hodgkin Lymphoma

| Limited Stage                                                         |                            | Advanced Stage                                                                                  |
|-----------------------------------------------------------------------|----------------------------|-------------------------------------------------------------------------------------------------|
| EORTC                                                                 | GHSG                       | International Collaborative Study                                                               |
| Adverse Prognostic Factors                                            |                            | Adverse Prognostic Factors                                                                      |
| MMR $\geq 0.35$                                                       | MMR $\geq 0.35$            | Age $\geq 45$ years                                                                             |
| ESR $> 30$ if symptomatic                                             | ESR $> 30$ if symptomatic  | Stage IV                                                                                        |
| ESR $> 50$ if asymptomatic                                            | ESR $> 50$ if asymptomatic | Male sex                                                                                        |
| $> 3$ Ann Arbor sites                                                 | $> 2$ Ann Arbor sites      | White blood count $\geq 15 \times 10^9/L$                                                       |
| Age $\geq 50$                                                         | Extranodal disease         | Lymphocyte count $< 0.6 \times 10^9/L$ or $< 8\%$                                               |
| Massive splenic disease                                               |                            | Albumin $< 4$ g/dL                                                                              |
| Presence of any factor is considered unfavorable                      |                            | Hemoglobin $< 10.5$ g/dL                                                                        |
| Two-thirds of limited-stage patients have one or more adverse factors |                            | Factors summed to yield the international prognostic score; 75% of patients have a score of 1–3 |

EORTC, European Organization for the Research and Treatment of Cancer; ESR, erythrocyte sedimentation rate; GHSG, German Hodgkin Study Group; MMR, mediastinal mass ratio, which is the ratio of the maximal width of a mediastinal mass relative to the maximal width of the mediastinum, as measured by computed tomographic imaging.

An independent data-monitoring committee stopped the study after a median followup of 1.1 years because of inferior results in the experimental group, with a 1-year progression-free survival of only 94 percent compared to 100 percent in patients treated in the standard arm ( $p = 0.017$ ). The independent data-monitoring committee mandated that all patients on the experimental arm be crossed over to receive involved nodal radiotherapy.<sup>101</sup> Interestingly, the opposite conclusion was reached by investigators in England conducting a similarly designed trial, dubbed the “RAPID” trial.<sup>102</sup> In this study, patients with early stage cHL were given three cycles of ABVD followed by a PET scan. Patients who were PET negative after three cycles of ABVD were randomized to receive either 30 Gy of involved-field radiotherapy or no further treatment. Patients who were PET positive after three cycles received a fourth cycle of ABVD followed by involved-field radiotherapy. An intent-to-treat analysis of the randomized PET-negative population revealed a 3-year progression-free survival of 94.5 percent in the combined modality group compared to 90.8 percent in those receiving only three cycles of ABVD ( $p = 0.23$ ). An analysis of the data according to the treatment actually received revealed a 3-year progression-free survival of 97 percent in the combined modality group compared to 90.7 percent in the ABVD-alone group ( $p = 0.03$ ). There were no statistically significant differences in OS between the groups (97.1 vs. 99.5 percent, respectively;  $p = 0.07$ ). The investigators conducting this trial concluded that the inferior progression-free survival in the group receiving ABVD alone was acceptable in order to avoid late complications associated with radiotherapy. In view of the conflicting conclusions derived from these two large phase III trials, the role of interim FDG-PET/CT imaging in early stage cHL remains controversial.

Nevertheless, the National Comprehensive Cancer Network has incorporated interim PET imaging into their guidelines, suggesting that early stage cHL patients who are PET negative after two cycles of ABVD may be treated with chemotherapy alone, without radiotherapy.<sup>158</sup>

## UNFAVORABLE LIMITED-STAGE HODGKIN LYMPHOMA

Patients with “unfavorable” prognostic factors (large tumor bulk, defined as a mass 10 cm or larger in diameter or more than one-third of the trans-thoracic diameter; an ESR of 50 or greater; three or more sites of tumor involvement; the presence of B symptoms; or the presence of extranodal sites; Table 8–4) may require more intensive treatment than do patients not exhibiting any of these features.<sup>158</sup> The EORTC and the Groupe d’Etude des Lymphomes de l’Adulte (GELA) reported results from a randomized study (H9U) in which such “unfavorable” early stage patients were randomized to treatment with either four or six cycles of ABVD or six cycles of BEACOPP, each followed by 30-Gy involved-field radiotherapy.<sup>156</sup> No significant differences were observed among these three treatment arms, establishing four cycles of ABVD and 30 Gy of involved-field radiotherapy as a standard of care for these patients. The GHSG subsequently randomized 1395 patients with unfavorable early stage Hodgkin lymphoma to four cycles of either ABVD or BEACOPP<sub>baseline</sub> followed by either 20 or 30 Gy of involved-field radiotherapy. In this study, four cycles of ABVD followed by 30 Gy of radiotherapy was the best approach, affording an 85 percent rate of “freedom from treatment failure” and an OS rate of approximately 95 percent after 5 years while maintaining a favorable toxicity profile.<sup>159</sup> The administration of only 20 Gy of radiotherapy following ABVD was found to be clearly inferior to the other three treatment arms, which all administered 30 Gy to this unfavorable group of patients. The subsequent GHSG HD14 study reported an advantage in progression-free survival for two cycles of escalated BEACOPP and two cycles of ABVD plus radiation therapy compared to four cycles of ABVD–radiation therapy, in a specified interim analysis, though no OS benefit was seen.<sup>160</sup> After 3 years, 90 percent of ABVD–radiation therapy patients were disease free compared to 96 percent treated with the BEACOPP–ABVD–radiation therapy regimen. The EORTC/LySA/FIL H10 trial randomized both favorable and unfavorable early stage patients to either standard therapy or experimental, PET-response adapted therapy.<sup>101</sup> The standard treatment was ABVD for four cycles followed by 30 Gy of involved nodal radiation therapy (INRT) versus PET-response adapted therapy. Patients in the experimental arm received six cycles of ABVD without any radiotherapy if the PET scan was negative after the second cycle of ABVD, whereas PET-positive patients were switched to BEACOPP<sub>escalated</sub> for two cycles followed by radiotherapy. For interim PET-positive patients, 5 year progression-free survival was 77.4 percent with standard ABVD plus INRT versus 90.6 percent for those switched to BEACOPP<sub>escalated</sub> plus INRT. For the interim PET-negative patients, 5-year progression-free survival rates for favorable patients was 99.0 percent versus 87.1 and 92.1 percent versus 89.6 percent—both favoring use of INRT—and noninferiority of chemotherapy alone was not demonstrated.<sup>101</sup>

## ADVANCED DISEASE

ABVD became the standard therapy for advanced Hodgkin lymphoma by proving to be superior to the MOPP chemotherapy and equal to, but less toxic than, hybrid or alternating combinations with MOPP.<sup>141,142,161–164</sup> Specifically, the incidence of secondary myelodysplasia, leukemia, and sterility was less with ABVD. The GHSG developed the BEACOPP regimen (Table 8–3) based upon mathematical modeling that indicated

a moderate increase in chemotherapy dose intensity would result in a significant increase in the cure rate. In the original HD9 study, BEACOPP was given in “standard” and “escalated” versions, the latter facilitated by granulocyte colony-stimulating factor use, and was compared with the cyclophosphamide, vincristine (Oncovin), procarbazine, prednisone (COPP)–ABVD regimen.<sup>143</sup> Patients with initial tumors equal to or greater than 5 cm or residual radiographic disease received 36-Gy radiotherapy after chemotherapy. The 5- and 10-year results demonstrated a significant progression-free and OS advantage for escalated BEACOPP compared to COPP-ABVD.<sup>143,144</sup> The cure rates in excess of 80 percent for escalated BEACOPP are the best ever recorded for a large phase III trial in advanced Hodgkin lymphoma. The superiority of escalated BEACOPP was observed regardless of the clinical international prognostic score.<sup>124</sup> Despite these outcomes, BEACOPP has not been universally accepted as the new standard in advanced Hodgkin lymphoma because of concerns about the acute toxicity, which includes greater need for hospitalization and transfusion, and late toxicity, which includes male and female sterility and an increased risk of secondary leukemia.<sup>165–168</sup> In addition, approximately two-thirds of patients received radiotherapy in the HD9 study. Two randomized clinical trials from Italy subsequently confirmed the superiority of BEACOPP over ABVD for the end point of progression-free survival.<sup>169,170</sup> In the first trial, BEACOPP (four escalated dose cycles plus two standard cycles) was compared to ABVD; all patients were to receive consolidation radiotherapy for bulky or residual disease. BEACOPP yielded significantly superior progression-free survival compared to ABVD, although no difference in OS was observed.<sup>169</sup> In the second study, patients were randomized to ABVD versus four cycles of standard and four cycles of escalated BEACOPP with preplanned retreatment and high-dose therapy and autologous transplantation for treatment failures.<sup>170</sup> This study also showed a significantly higher progression-free survival for the BEACOPP arm but no difference in OS (Table 8–5). The EORTC 20012 trial randomized patients with “high-risk” advanced-stage Hodgkin lymphoma with an international prognostic scale score of 3 to 7<sup>124</sup> to treatment with either BEACOPP (four escalated cycles + four standard cycles) or ABVD.<sup>171</sup> Progression-free survival was improved in the BEACOPP arm, but there was no statistically significant improvement in event-free survival or OS compared to ABVD.<sup>171</sup> In each of the four cited trials, the hazard ratio for relapse with BEACOPP was approximately 0.5. Standards of care in advanced Hodgkin lymphoma continue to be debated in view of the lack of a survival benefit with the BEACOPP program, which benefits approximately 15 percent of patients while exposing 100 percent of patients to more toxicity.

Efforts to reduce the risk of treatment-related morbidity of escalated BEACOPP have included studying the combination of four cycles of escalated and four cycles of standard BEACOPP and eliminating radiation therapy. In the GHSG HD12 trial, four standard cycles of BEACOPP plus four escalated cycles performed similarly to eight cycles of escalated BEACOPP, with freedom from treatment failure rates of 85 and 86 percent, respectively, 5 years after treatment. In this study, there was a slight trend for worse failure-free survival in patients with residual radiographic masses on end-of-treatment CT imaging who did not receive consolidative radiotherapy (90 percent after 5 years with radiotherapy and 87 percent without it,  $p = 0.08$ ).<sup>172</sup> The subsequent HD15 trial randomized 2182 patients with newly diagnosed advanced-stage cHL to receive either eight cycles of BEACOPP<sup>escalated</sup> or six cycles of BEACOPP<sup>escalated</sup> or eight cycles of the BEACOPP-14 regimen. Patients with a persistent mass measuring 2.5 cm or larger after chemotherapy that was FDG avid on an end-of-treatment FDG-PET imaging received consolidative radiotherapy (30 Gy). Freedom from treatment failure was similar for all three treatment arms, but 5-year OS was significantly better for patients receiving six cycles of BEACOPP<sup>escalated</sup> than for

8 cycles of BEACOPP<sup>escalated</sup> (91.9 vs. 95.3 percent) as a result of higher treatment-related mortality and secondary malignancies with the latter regimen.<sup>173</sup>

Numerous investigations are under way to determine if interim PET scans can direct more intensive treatment with escalated BEACOPP to the subgroup that benefits.<sup>95,97,100,174,175</sup> Likewise, interim and end-of-treatment PET scans are being used to direct the use of consolidative radiotherapy.<sup>95,174–177</sup> Although there is great enthusiasm regarding this approach to direct subsequent treatment, it is notable that the majority of patients remaining PET positive after four cycles of escalated BEACOPP appear to be cured by the planned therapy, in contrast to findings with ABVD.<sup>177</sup>

In the Response Adapted Therapy for Hodgkin Lymphoma (RATHL) study, interim PET-CT was also employed to modify therapy.<sup>177a</sup> Patients with advanced-stage cHL received two cycles of ABVD and then underwent a PET-CT scored by a 5-point scale. Those with positive PET went on to receive BEACOPP for four cycles, demonstrating a 3-year progression-free survival of 67.5 percent and OS of 87.8 percent. Those with negative interim PET-CT were randomized to four additional cycles of either ABVD or AVD (without bleomycin). In this group, 3-year progression-free survival was 85.7 percent and 84.4 percent, with similar OS (97.2 and 97.6 percent). Given the similar efficacy and less frequent respiratory toxicity, these data suggest that advanced-stage HL patients who are FDG-PET negative after two cycles of ABVD can omit bleomycin from cycles 3–6 without significantly compromising treatment efficacy.

The Stanford group took an alternate approach to advanced Hodgkin lymphoma by abbreviating the duration of therapy and reducing cumulative drug doses in the Stanford V regimen.<sup>178</sup> This approach appeared highly successful in institutional and phase II cooperative group trials; however, three randomized controlled trials have failed to show improved progression-free survival compared to standard ABVD chemotherapy.<sup>179–181</sup> The impressive efficacy of brentuximab vedotin in relapsed and refractory cHL<sup>182,183</sup> has also led to investigations into its incorporation into frontline regimens. This drug consists of an anti-CD30 monoclonal antibody conjugated to a highly potent antitubulin cytotoxic drug, monomethyl auristatin E. Phase I studies revealed unacceptable pulmonary toxicity when brentuximab vedotin was incorporated into the full ABVD regimen,<sup>184</sup> presumably as a result of augmenting the pulmonary toxicity of bleomycin. Omission of bleomycin, however, allows safe and effective combination therapy with brentuximab vedotin concurrently with AVD, with 96 percent of patients achieving complete remission.<sup>184</sup> An international phase III randomized comparison of standard ABVD versus brentuximab vedotin plus AVD is under way for patients with advanced cHL.

The use of radiotherapy as consolidation after combination chemotherapy in advanced cHL is controversial. Encouraging data from single institutions in adults and children were not borne out in randomized trials, some of which were criticized as underpowered. Furthermore, chemotherapy regimens have evolved over the time span of these studies. The application of 30-Gy involved-field radiotherapy to patients in complete remission after MOPP-ABV was studied in an adequately powered phase III trial.<sup>185</sup> No significant difference in failure-free survival was observed. Of note, all patients in partial remission received 40 Gy on this study, and their outcome was not different from complete remission patients. The GHSG HD12 study randomized patients to observation or consolidation radiotherapy, with the incorporation of a central review panel, following BEACOPP. The final analysis of this study reported a slight decrease in the freedom from treatment failure if radiotherapy was omitted in patients who had persistent radiographic abnormalities on CT imaging after chemotherapy (90.4 vs. 87 percent after 5 years), but no difference was seen in patients with bulk disease in complete

**TABLE 8-5.** Selected Randomized Clinical Therapeutic Trials in Hodgkin Lymphoma

| Study (Number of Patients)               | Treatment                                 | Failure-Free Survival (%) | Overall Survival (%) | Followup (Years) |
|------------------------------------------|-------------------------------------------|---------------------------|----------------------|------------------|
| LIMITED STAGE, FAVORABLE AND UNFAVORABLE |                                           |                           |                      |                  |
| Milan (140) <sup>149</sup>               | 4 ABVD + IFRT                             | 94                        | 94                   | 12               |
|                                          | 4 ABVD + STLI                             | 93                        | 96                   |                  |
|                                          |                                           | <i>p</i> = NS             | <i>p</i> = NS        |                  |
| NCIC-ECOG (405) <sup>155</sup>           | STLI                                      | 92                        | 87                   | 12               |
|                                          | 4–6 ABVD                                  | 87                        | 94                   |                  |
|                                          |                                           | <i>p</i> = 0.006          | <i>p</i> = NS        |                  |
| LIMITED STAGE, FAVORABLE                 |                                           |                           |                      |                  |
| EORTC/GELA H9F (783) <sup>156</sup>      | 6 EBVP + 20-IFRT                          | 84                        | 98                   | 4                |
|                                          | 6 EBVP + 36-IFRT                          | 87                        | 98                   |                  |
|                                          | 6 EBVP                                    | 70                        | 98                   |                  |
|                                          |                                           | <i>p</i> ≤ 0.001          | <i>p</i> = NS        |                  |
| GHSG HD10 (1370) <sup>278</sup>          | 2 ABVD + 30-IFRT                          | 86                        | 94                   | 8                |
|                                          | 2 ABVD + 20-IFRT                          | 86                        | 95                   |                  |
|                                          | 4 ABVD + 30-IFRT                          | 87                        | 94                   |                  |
|                                          | 4 ABVD + 20-IFRT                          | 90                        | 95                   |                  |
|                                          |                                           | <i>p</i> = NS             | <i>p</i> = NS        |                  |
| LIMITED STAGE, UNFAVORABLE               |                                           |                           |                      |                  |
| EORTC/GELA H9U (808) <sup>156</sup>      | 6 ABVD + 30-IFRT                          | 91                        | 95                   | 4                |
|                                          | 4 ABVD + 30-IFRT                          | 87                        | 94                   |                  |
|                                          | 4 BEACOPP + 30-IFRT                       | 90                        | 93                   |                  |
|                                          |                                           | <i>p</i> = NS             | <i>p</i> = NS        |                  |
| GHSG HD11 (1422) <sup>159</sup>          | 4 ABVD + 30-IFRT                          | 85                        | 94                   |                  |
|                                          | 4 ABVD + 20-IFRT                          | 81*                       | 94                   |                  |
|                                          | 4 BEACOPP + 30-IFRT                       | 87                        | 95                   |                  |
|                                          | 4 BEACOPP+ 20-IFRT                        | 87                        | 95                   |                  |
| ADVANCED STAGE                           |                                           |                           |                      |                  |
| GHSG HD9 (1201) <sup>143</sup>           | 8 COPP/ABVD + RT                          | 64                        | 75                   | 10               |
|                                          | 8 BEACOPP + RT                            | 70                        | 80                   |                  |
|                                          | 8 BEACOPP <sub>escalated</sub> + RT       | 82                        | 86                   |                  |
|                                          |                                           | <i>p</i> < 0.0001         | <i>p</i> < 0.005     |                  |
| US Intergroup (854) <sup>181</sup>       | 6–8 ABVD                                  | 74                        | 88                   | 5                |
|                                          | Stanford V                                | 71                        | 88                   |                  |
|                                          |                                           | <i>p</i> = NS             | <i>p</i> = NS        |                  |
| Italian Intergroup (331) <sup>170</sup>  | 6–8 ABVD                                  | 73                        | 84                   | 7                |
|                                          | BEACOPP (4 escalated + 4 standard cycles) | 85                        | 89                   |                  |
|                                          |                                           | <i>p</i> = 0.004          | <i>p</i> = 0.39      |                  |

ABVD, doxorubicin, bleomycin, vinblastine, dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone; COPP, cyclophosphamide, vincristine, procarbazine, prednisone; EBVP, epirubicin, bleomycin, vinblastine, prednisone; ECOG, Eastern Cooperative Oncology Group; EORTC, European Organization for the Research and Treatment of Cancer; GELA, Groupe d'Etude des Lymphomes de l'Adulte; GHSG, German Hodgkin Study Group; IFRT, involved-field radiotherapy; NCIC, National Cancer Institute of Canada; RT, radiotherapy; STLI, subtotal lymphoid irradiation.

\* $p = 0.03$ .



response after chemotherapy. Unfortunately, FDG-PET imaging was not routinely used in this trial.<sup>172</sup> The two Italian studies comparing ABVD with BEACOPP mentioned previously routinely incorporated consolidation radiation therapy for residual or bulky disease.<sup>169,186</sup> The HD15 study limited the use of radiotherapy to patients with positive PET scans after four cycles of chemotherapy. With this approach, only 12 percent of patients received radiotherapy, and the progression-free survival rate among PET-negative patients, who did not receive radiotherapy, was 96 percent after 1 year.<sup>173</sup> In sum, the data do not support the routine use of radiotherapy following a full course of chemotherapy in advanced Hodgkin lymphoma. However, the role of this potent treatment in patients with an early incomplete response or following a brief course of chemotherapy is likely to be more significant.<sup>177,178</sup>

## NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

NLPHL presents as asymptomatic, limited-stage disease in most (~80 percent) patients.<sup>103,104,109</sup> Peripheral lymph nodes in the neck, axilla, or groin are commonly involved as stage IA disease. The European Task Force on Lymphoma reported a 96 percent complete response rate and 99 percent and 94 percent 8-year disease-specific survival for stages I and II disease, respectively.<sup>109</sup> Because of the low likelihood of occult disease in NLPHL and the tendency for the disease to remain localized for years, regional radiation therapy is considered the treatment of choice for early stage disease.<sup>104</sup> Analyses from the GHSG demonstrate that outcomes with limited radiation therapy are comparable to the use of more extensive radiation and combined modality regimens.<sup>187</sup> For the 20 percent of patients who present with stage III or IV disease, most authorities advise treatment with ABVD-based chemotherapy following the paradigms developed for cHL,<sup>104</sup> although some contend that alkylator-based regimens such as rituximab, cyclophosphamide, hydroxydaunorubicin, vincristine (Oncovin), prednisone (R-CHOP) may be equally effective and less toxic.<sup>188</sup> Because of the consistent high-level expression of the CD20 antigen on the surface of NLPHL cells, the monoclonal antibody rituximab has been tested in this entity. Initial studies were conducted using single-agent rituximab in relapsed and refractory cases and demonstrated response rates of 94 to 100 percent with median durations of remission of 33 to 60 months, with longer remissions observed when maintenance rituximab was used.<sup>189,190</sup> Based on these findings, and the low toxicity of rituximab, some authorities have extrapolated its use to the frontline setting,<sup>188</sup> adding it to radiotherapy for stage I or II patients and to ABVD for stage III or IV disease, although few data have been published concerning the frontline use of this agent in NLPHL.

## RECURRENT DISEASE

Historically, patients with cHL who relapsed after a full course of chemotherapy had a low chance for cure with second-line treatment, with the duration of initial remission a significant predictor of subsequent response and relapse-free survival. High-dose therapy and autologous blood stem cell transplantation improved the outlook for such patients and is routinely employed in first relapse for most patients younger than age 65 years, based on institutional and phase III trial experience.<sup>191,192</sup> Cure rates with transplantation range from 40 to 60 percent, with transplant-related mortality less than 5 percent.<sup>193,194</sup> High-dose regimens include BEAM (carmustine, etoposide, cytarabine, melphalan); CBV (cyclophosphamide, carmustine, etoposide); and total-body irradiation with cyclophosphamide and etoposide. Consolidation radiotherapy is often employed to sites of pretransplantation bulk disease. In unfavorable risk patients (defined by partial vs. complete response to

second-line therapy and time to relapse from frontline therapy of <12 months), 16 cycles (every 3 weeks) of brentuximab vedotin after autologous stem cell transplantation was associated with a progression-free survival benefit. Toxicity consisted primarily of sensory neuropathy and neutropenia, and no OS benefit was demonstrated.<sup>194a</sup> The superiority of any single transplant conditioning regimen has not been definitively established; however, the use of high-dose sequential therapy coupled with tandem autologous transplantation is being tested in randomized trials.<sup>195,196</sup> In most cases, second-line chemotherapy with ICE (ifosfamide, carboplatin, etoposide); GVD (gemcitabine, vinorelbine, liposomal doxorubicin); DHAP (dexamethasone, cytarabine, cisplatin); gemcitabine, dexamethasone, cisplatin (GDP); or brentuximab vedotin is used to achieve a minimal disease state prior to stem cell mobilization and transplantation.<sup>197–199</sup> Numerous combinations of brentuximab vedotin with chemotherapy or novel agents are under evaluation. Treatment failures following autologous transplantation present a challenge, with longevity directly related to the time to relapse after transplantation. Allogeneic transplantation in multiply recurrent Hodgkin lymphoma has been limited by significant transplant-related mortality, although long-term disease control has been observed in a small subset together with anecdotal evidence of a graft-versus-Hodgkin anti-tumor effect. Nonmyeloablative transplantation conditioning regimens reduce transplant-related mortality, but disease recurrence continues to present a major challenge, with failure-free survivals in the 20 to 30 percent range.<sup>199–201</sup>

As mentioned, the anti-CD20 antibody rituximab achieves high response rates in relapsed NLPHL and can be used as re-treatment or as an extended-treatment regimen.<sup>189,202</sup> Monoclonal antibodies directed against the CD30 antigen are well tolerated in cHL but have limited therapeutic value.<sup>203</sup> However, the anti-CD30 antibody–drug conjugate brentuximab vedotin has major activity, with 96 of 102 patients with relapsed or refractory cHL experiencing tumor shrinkage in a phase II clinical trial, including 75 percent with objective remissions and 34 percent with complete remissions.<sup>183</sup> The agent has also shown major efficacy when used as a bridge to allogeneic transplantation<sup>204</sup> or in patients relapsing after allogeneic stem cell transplantation.<sup>205</sup> The major toxicity of this agent when used as a single agent is peripheral neuropathy, though mild-to-moderate cytopenias also occur. The malignant cells in cHL are associated with genetic alterations of the 9p24.1 locus, resulting in immune evasion due to overexpression of PD-1 ligands. Nivolumab and pembrolizumab are PD-1–blocking antibodies that have recently been shown to have remarkable efficacy in patients with relapsed or refractory cHL, with objective remission rates of up to 87 percent observed in trials enrolling heavily pretreated patients, including those who had received prior brentuximab vedotin.<sup>205a–205d</sup> Ongoing studies are evaluating combinations of immune checkpoint inhibitors with each other or with brentuximab vedotin in the relapsed setting, as well as in the upfront disease setting in conjunction with standard treatments.

## COURSE AND PROGNOSIS

The goal of treatment for Hodgkin lymphoma is to cure the greatest number of patients with the fewest complications. Improvements in management have resulted in cure for a large majority of patients younger than age 65 years. Survival expectations at 10 years for patients diagnosed from 2006 to 2010 exceed 90 percent for patients to age 44 years, 80 percent for patients to age 54 years, and 70 percent for patients to age 64 years.<sup>206</sup> These outstanding results have been achieved by refining the use of radiotherapy and chemotherapy in the frontline setting and the development of improved secondary treatments for patients with persistent or relapsed disease. However, the late effects of

treatment for Hodgkin lymphoma remain a concern for cured patients, and a small subset of patients has refractory disease.

## CLINICAL PROGNOSTIC FACTORS

A number of complex prognostic factor schemes have been developed for limited Hodgkin lymphoma treated with radiotherapy alone (Table 8–4). Massive mediastinal disease and constitutional symptoms have been consistently identified as independent predictors of relapse, whereas only older age was predictive of inferior survival. European and Canadian investigators incorporated gender, age, ESR, number of Ann Arbor disease sites, stage, and histology into stratifications for favorable, very favorable, and unfavorable disease categories. The EORTC defines four or more nodal sites, ESR greater than 50 in asymptomatic patients or ESR greater than 40 in symptomatic patients, and histology as indicators of intermediate disease, whereas the GHSG designates any one of the following: massive mediastinal disease, extranodal disease, ESR greater than 50 if asymptomatic and greater than 30 if symptomatic, and three or more nodal sites as intermediate disease (Table 8–4). It is important to be aware of the variable eligibility criteria when interpreting the literature in early stage Hodgkin lymphoma and to note that these clinical variables are currently used to group patients for clinical investigations. The international prognostic score, based on seven factors (see Table 8–4), is used in advanced disease.<sup>124,207</sup> Each factor reduced the freedom from progression by approximately 7 percent. Only 7 percent of patients were in the worst prognostic group (five to seven factors), and the freedom from progression in this subset was 42 percent at 5 years. Consensus with regard to prognostic factors promotes uniformity in clinical trial design and provides a rationale for alternate approaches in high-risk subsets. The superiority of escalated BEACOPP was seen across the spectrum of the international prognostic score, but approximately 30 percent of high-risk (four to seven factors) patients experienced treatment failure.<sup>144</sup> Alternately, the improvement in progression-free survival with BEACOPP over ABVD in an Italian study was limited to higher risk patients.<sup>169</sup> Age has been a consistent adverse prognostic marker regardless of tumor burden, but recent gains eliminate this adverse factor up to approximately age 55 years.<sup>208,209</sup> Although less-intensive treatment may explain inferior results in a subset of patients, results in older patients are worse, even when the intensity of therapy is considered.<sup>209</sup> The BEACOPP regimen was associated with unacceptable toxicity and demonstrated no advance over ABVD for patients older than age 65 years.<sup>210</sup> Various groups are exploring alternative treatment regimens for older patient populations, including the prednisone, vinblastine, doxorubicin, gemcitabine (PVAG) regimen and brentuximab vedotin–based approaches, in order to minimize toxicity.<sup>210a,210b</sup>

Normal results from FDG-PET imaging at the completion of treatment confer a high negative predictive value, ranging from 81 to 100 percent.<sup>176</sup> The positive predictive value at the end of chemotherapy is more variable and is related to disease extent and use of radiotherapy.<sup>176,211,212</sup> There is great interest in FDG-PET imaging after one to three cycles of chemotherapy, as several studies indicated that negative results predict treatment success whereas positive results predict a high likelihood of treatment failure.<sup>95,96,98,99</sup> Numerous ongoing phase III clinical trials are designed to alter treatment based on early PET results with a goal of achieving high cure rates and minimizing toxicity.<sup>100</sup> PET status prior to autologous transplantation has also emerged as a dominant prognostic factor.<sup>213</sup>

Adverse prognostic factors identified for patients with relapsed cHL undergoing autologous stem cell transplantation include (1) duration of first complete remission less than 1 year, (2) failure to achieve a second complete remission with salvage chemotherapy prior to

transplantation, (3) presence of B symptoms or extranodal sites of disease involvement, and (4) persistence of FDG avidity on PET imaging prior to transplant.<sup>213–215</sup>

Patients with primary progressive Hodgkin lymphoma have the least favorable prognosis. Fortunately, newer treatment approaches have reduced the proportion of patients in this category.

Clinical prognostic factors are surrogates for the underlying cellular and molecular biology of Hodgkin lymphoma. Serum levels of cytokines, including soluble CD30, a probable marker of tumor burden, and IL-10, a measure of immunosuppression related to the microenvironment, are associated with adverse prognosis independent of clinical features.<sup>216</sup> The chemokine CCL17, secreted by Hodgkin and Reed-Sternberg cells, is elevated in patients' sera and is being studied as a marker of response.<sup>217,218</sup> Multiple, but not all, investigators found BCL-2 expression to be of prognostic significance.<sup>219–222</sup> CD20 expression by Hodgkin and Reed-Sternberg cells has been associated with less-favorable outcomes in some, but not all, series.<sup>223,224</sup> Lack of HLA class II expression, which may allow immune escape of Hodgkin and Reed-Sternberg cells, was found to be an independent prognostic factor.<sup>225</sup> The prognostic significance of EBV association varies with age, conferring an adverse outcome in older individuals.<sup>226,227</sup> Furthermore, single nucleotide polymorphisms in HLA-A2 have been associated with risk of EBV-positive Hodgkin lymphoma.<sup>52</sup> A number of studies have focused on the inflammatory microenvironment of Hodgkin lymphoma. Increased numbers of T-regulatory cells have correlated with favorable outcomes, and decreased numbers of markers for cytotoxic T cells have correlated with adverse outcomes in several series.<sup>228–230</sup> Similarly, heavy infiltration of cHL with macrophages (detected by immunohistochemical staining for CD68) is associated with poor outcome, presumably because of secretion of immunosuppressive cytokines.<sup>231</sup> Finally, a 23-gene expression signature that can be assessed in formalin-fixed, paraffin-embedded tissue biopsies has been suggested to have prognostic power in patients with cHL.<sup>232</sup> Together, these findings suggest an important interplay between characteristics of Hodgkin and Reed-Sternberg cells and the inflammatory environment.

## COMPLICATIONS OF TREATMENT

The treatment of Hodgkin lymphoma is associated with important acute and chronic side effects. Although the acute complications of chemotherapy and radiotherapy may be troublesome, they are relatively easily managed. Late-treatment effects in the form of sterility, second malignancy, and cardiopulmonary disease are more serious and are known to contribute to shortened longevity for cured patients.<sup>145,152,233,234</sup> Excess mortality from second malignancies and cardiac disease increase with time and are currently the leading causes of death for cHL patients. As treatment has evolved, the risks of radiation-related complications has lessened, but long latency periods and uncertainty regarding associations with lower doses make it difficult to predict individual risks. Recognition and understanding of these problems help to shape primary treatment choice and facilitate optimal followup for survivors.

Acute leukemia and myelodysplasia were the initial second malignancies to be observed after successful treatment for Hodgkin lymphoma with MOPP chemotherapy.<sup>235</sup> The risk following MOPP was proportional to the cumulative dose of alkylating agents and was associated with recurring abnormalities of chromosomes 5 and 7.<sup>235–237</sup> Actuarial risks of approximately 5 percent with relative risks in excess of 100 have been reported over a 7- to 10-year period with alkylating agent–based therapies. The risk of secondary leukemia is greater in patients older than age 35 years. Prognosis for secondary leukemia is poor, with survivals of less than 1 year.<sup>238</sup> The risk of acute leukemia is significantly less after ABVD chemotherapy,<sup>141</sup> although it is not absent. A large

international study observed a significant reduction in excess absolute risk after 1984, presumably as a consequence of change in primary therapy.<sup>238</sup> However, acute leukemia may complicate Hodgkin lymphoma treatment with higher doses of etoposide and doxorubicin, such as in the BEACOPP regimen.<sup>143,165,168</sup> This form of leukemia tends to occur earlier and be associated with balanced translocations of chromosome 11. Patients who have received second-line therapies and autologous transplantation are at highest risk for myelodysplasia and secondary leukemia.

There is an increased relative risk of non-Hodgkin lymphomas after treatment for Hodgkin lymphoma.<sup>239,240</sup> These are diffuse, aggressive B-cell lymphomas that may occur early or late after treatment. There is no clear relationship to the type of primary treatment. The incidence of secondary lymphoma in a series of 5406 patients treated on GHSG protocols was 0.9 percent; prognosis was worse if lymphoma developed within 3 months of primary therapy.<sup>241</sup> Although prognosis was relatively poor in this series, the data antedated the routine use of rituximab in treatment regimens. It is not clear how non-Hodgkin lymphomas relate to treatment-related immunodeficiency, predisposition to B-cell malignancy, or a shared common cell of origin. Marginal zone lymphomas with identical B-cell receptor genes also have been identified.<sup>242</sup> Diffuse large B-cell lymphoma and its variants are most frequent in NLPHL, where they have been found to be genetically related.<sup>243</sup>

An increased risk of solid cancers after treatment for Hodgkin lymphoma has long been recognized, and with time, these have emerged to account for 75 to 80 percent of all cases of second malignancy.<sup>145,152,239</sup> The risk is related to radiotherapy exposure, with tumors occurring in or at the edges of the radiation field. The most common solid tumors are breast, lung, and gastrointestinal malignancies. The latency for developing second cancers is an important consideration, as these typically develop after at least 10 years and continue to pose excess risk for as long as 30 years after treatment. Breast cancer is increased in women treated before age 30 years and is markedly increased in children and adolescents.<sup>152,244–246</sup> Case-control studies have examined the relationship of radiation dose to the breast and the risk of cancer, finding a 3.2-fold increased risk with doses greater than 4 Gy and an eightfold risk for doses greater than 40 Gy.<sup>247,248</sup> Elimination of routine axillary radiation, which is now standard, results in a 2.7-fold reduction in risk, and it is anticipated that risks may further decline with current low-dose or nodal radiotherapy. Cofactors are important for defining the risks of second breast cancer, which are highest for women younger than age 30 years when irradiated and for those who continue to have normal menses.<sup>249,250</sup>

Lung cancer risk is greatest among patients who are older than 45 years of age when treated. Tobacco exposure has a multiplying effect, and alkylating agent exposure also contributes to risk. Among patients with chest irradiation, a tobacco history, and alkylating chemotherapy, the lung cancer risk was 49-fold higher than in patients who had none of these exposures.<sup>248</sup> Alkylating agent chemotherapy independently increases risk of lung cancer with dose-response associations reported in population-based studies.<sup>247,248,251</sup>

Estimates of relative risks of cardiac mortality in Hodgkin lymphoma survivors range from 2.2- to sevenfold.<sup>233,234,252,253</sup> Mediastinal radiotherapy is associated with an increased risk of cardiac disease. An increased risk of death from coronary artery disease and acute myocardial infarction has been identified in adults and children.<sup>245,253,254</sup> Other types of cardiac disease are often asymptomatic, including valvular disease, conduction defects, and cardiomyopathy.<sup>255,256</sup> The risks of radiation-related heart disease do not appear to be influenced significantly by the addition of chemotherapy. The onset of increased risk is within 5 to 10 years. As risk is associated with the dose and volume of radiotherapy and the latency is 5 to 10 or more years, the hazards

associated with current lower dose and smaller fields remain to be assessed.<sup>252</sup> Established cardiac risk factors, including hypertension, hypercholesterolemia, and smoking, significantly contribute to the subsequent risk of cardiac disease after therapy, offering opportunities to reduce individual risks.<sup>257</sup> A British report indicated an elevated risk of cardiac mortality of 7.8-fold following ABVD alone, which rose to 12.1-fold when given with mediastinal irradiation.<sup>258</sup> These results provide a cautionary note, but more data are needed, and these results do not address cumulative exposures that are lower with modern therapy.

Noncoronary vascular complications have been reported after neck irradiation, with associations to dose greater than 36 Gy and cofactors of hypertension, diabetes, and hypercholesterolemia.<sup>259,260</sup> In a retrospective cohort study, the standardized incidence ratio was 2.2 for stroke and 3.1 for transient ischemic attack.<sup>259</sup> However, it is important to note that modern approaches to Hodgkin lymphoma therapy use lower radiation doses, smaller fields, and planning techniques that limit dose inhomogeneity and hot spots commonly seen in the neck area with older techniques.

Approximately 90 percent of males are permanently sterilized by six cycles of MOPP chemotherapy.<sup>261</sup> The risk is related to the cumulative dose of alkylating agents, such that two to three cycles of MOPP result in azoospermia in approximately 50 percent of patients.<sup>262</sup> Female fertility after alkylating agent-based treatment is related to age at treatment as well as cumulative alkylating agent dose.<sup>263,264</sup> The ABVD combination is associated with temporary amenorrhea and azoospermia, with full recovery noted in 50 to 95 percent of patients.<sup>265,266</sup> A case-control study found no significant reduction in fertility among women treated with ABVD.<sup>267</sup> In contrast, no men had normospermia following treatment with BEACOPP, and amenorrhea occurred in more than 50 percent of women.<sup>166,167,268</sup> Several authors have described pregnancy outcome following treatment for Hodgkin lymphoma. No increase in birth defects or complications of pregnancy has been seen.<sup>264</sup>

Thyroid dysfunction is common after neck irradiation, reaching a risk of 47 percent at 26 years in the Stanford series.<sup>269</sup> Thus, patients at risk should be monitored during followup observation. Rarely, hyperthyroidism, Graves ophthalmopathy, or thyroid neoplasms occur after neck radiotherapy.<sup>269</sup> Lhermitte sign, a transient complaint of an “electric shock” sensation produced by head flexion, is a common sequela of mantle radiotherapy.<sup>270</sup> The incidence of radiation pneumonitis depends on the volume of lung irradiated and the total dose. Symptoms include cough, dyspnea, and fever. Although prospective assessment of pulmonary function demonstrates reduction of lung volumes following mantle radiotherapy, recovery is seen in 12 to 24 months, and symptomatic radiation pneumonitis is unusual.<sup>271,272</sup>

Full-dose radiation therapy interferes with normal growth and development in children. Current therapy programs use low-dose or no radiotherapy for all stages of disease. Overwhelming sepsis is a rare event in patients who have been splenectomized and treated for Hodgkin lymphoma, particularly children.<sup>273,274</sup> Vaccination against encapsulated organisms 10 to 14 days prior to the onset of treatment is advised. However, it must be recognized that neither vaccines nor antibiotic prophylaxis may provide adequate protection. Fatigue is commonly reported in Hodgkin lymphoma survivors and has been related to pulmonary function and peak oxygen uptake.<sup>255,275</sup>

With the high rates of cure currently attained in the management of Hodgkin lymphoma, reduction in late effects and quality of life assume even greater importance. Patient education is essential to promote healthy behaviors to reduce modifiable risk factors. In addition, early detection and prevention strategies for second cancers and cardiac disease should be considered in high-risk patients. However, the choice and efficacy of diagnostic testing, and their optimal timing and frequency, require further study.<sup>276</sup> Most of the documented late effects



relate to outdated chemotherapy and radiotherapy protocols, and that modeling, as well as recent data, indicate that lesser exposures significantly reduce second cancer risk.<sup>249,277</sup> Modern therapies will likely further reduce the risks of late complications. It continues to be important to follow long-term survivors, and the contribution of genetic and environmental factors is an important ongoing area of inquiry.

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## CHAPTER 9

# DIFFUSE LARGE B-CELL LYMPHOMA AND RELATED NEOPLASMS

Stephen D. Smith and Oliver W. Press\*

### SUMMARY

Diffuse large B-cell lymphomas (DLBCLs) comprise a heterogeneous group of aggressive malignancies of large, transformed B lymphocytes. DLBCL is the most common lymphoma in the world and accounts for approximately 25 to 30 percent of lymphoma cases in the United States. The incidence increases with age, with a median age at presentation in the sixth decade. The disease typically presents as a rapidly growing mass that may involve either lymph node or extranodal sites and often is associated with systemic symptoms. Approximately 50 to 60 percent of patients will present with advanced-stage, disseminated disease. DLBCL is curable with combination chemotherapy. For localized disease, either three cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) plus involved-field radiation therapy or six cycles of (R-CHOP) are recommended, whereas for advanced-stage DLBCL, six cycles of R-CHOP are appropriate. High-dose chemotherapy with autologous stem cell transplantation may be curative for patients with DLBCL that relapses after treatment with frontline chemotherapy.

**Acronyms and Abbreviations:** ABC, activated B-cell–like; ACVBP, doxorubicin (Adriamycin), cyclophosphamide, vindesine, bleomycin, prednisone; allo-HSCT, allogeneic hematopoietic stem cell transplantation; ASCT, autologous stem cell transplantation; BEAM, carmustine (bischloroethylnitrosourea), etoposide, cytarabine (Ara C), and melphalan; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CR, complete remission; CytaBOM, cytarabine, bleomycin, vincristine, methotrexate (with leucovorin rescue); DFS, disease-free survival; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; EFS, event-free survival; EPOCH, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin; ESHAP, etoposide, methylprednisolone, cytarabine, cisplatin; FDG, 18-fluorodeoxyglucose; GCB, germinal center B-cell–like; GELA, Groupe d'Etude des Lymphomes de l'Adulte; GVHD, graft-versus-host disease; ICE, ifosfamide, carboplatin, etoposide; IFRT, involved-field radiation therapy; Ig, immunoglobulin; LDH, lactate dehydrogenase; MACOP-B, high-dose methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin; m-BACOD, moderate-dose methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone; MOPP, mechlorethamine, vincristine (Oncovin), procarbazine, prednisone; NOS, not otherwise specified; OS, overall survival; PFS, progression-free survival; ProMACE, prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide; PTLD, posttransplantation lymphoproliferative disorder; R-CHOP, rituximab plus CHOP; R-EPOCH, rituximab plus EPOCH; R-ICE, rituximab plus ICE; VACOP-B, vincristine, doxorubicin, cyclophosphamide, etoposide, prednisone, and bleomycin; WHO, World Health Organization.

### DEFINITION AND HISTORY

Diffuse large B-cell lymphomas (DLBCLs) comprise a heterogeneous group of aggressive malignancies of large, transformed B cells, which cause diffuse effacement of the normal lymph node structure. DLBCL masqueraded under a variety of colorful but misleading monikers in early lymphoma classification systems, including “reticulum cell sarcoma” and “diffuse histiocytic lymphoma,” as described in an excellent review of the history of the lymphomas.<sup>1</sup> Distinct disease entities are distinguished based on morphologic, biologic, and clinical features as established by an international panel of experts on behalf of the World Health Organization (WHO; [Table 9–1](#)).<sup>2</sup> The disease can arise *de novo* or may transform from an indolent lymphoma, such as a small lymphocytic lymphoma or follicular lymphoma.

### EPIDEMIOLOGY

DLBCL is the most common B-cell lymphoid neoplasm in the United States and Europe and accounts for approximately 28 percent of all mature B-cell lymphomas.<sup>3,4</sup> Incidence varies by ethnicity, with Americans of European descent being more likely to develop DLBCL than Americans of African descent. Like most other lymphomas, there is a male predominance. The disease most commonly presents in late middle-aged and older persons, with a median age at diagnosis of approximately 65 years. Because lymphoma incidence rates increased dramatically from the 1940s to the 1990s, numerous exogenous factors have been examined to ascertain if one or more play a role in pathogenesis of the disease, including herbicides (e.g., phenoxyacids), pesticides (e.g., organochlorines), organic solvents (e.g., toluene, benzene), dark hair dyes, body mass index, tobacco use, alcohol use, and inflammatory states. At this time, no inhalant, exposure, or ingestant has been unequivocally proven to increase the relative risk of DLBCL.<sup>5</sup>

### ETIOLOGY AND PATHOGENESIS

DLBCL is a molecularly heterogeneous disease with multiple complex chromosomal translocations and genetic abnormalities as identified by cytogenetics, gene-expression profiling, and whole-genome sequencing. The disease is derived from B cells whose immunoglobulin (Ig) genes have undergone somatic mutation in the lymph node germinal center.<sup>6</sup> Approximately 40 percent of cases in immunocompetent hosts and approximately 20 percent of HIV-related cases display *BCL6* rearrangements.<sup>7–9</sup> Chromosomal translocations involving band 3q27 lead to a truncated *BCL6* gene within its 5' flanking region. Such truncations commonly occur within the first exon or first intron, leading to complete removal or truncation of the promoter sequences; the coding sequence is left intact.<sup>10</sup> In a small number of cases, the breakpoint is not located in the immediate proximity of the *BCL6* gene. Increased expression of *BCL6* occurs from a process termed *promoter substitution* by which heterologous promoters are juxtaposed to the *BCL6* coding domain. This process occurs through reciprocal translocations between 3q27 and chromosomal partner sites, including 14q32 (IgH), 2p11 (Igκ), and 22q11 (Igλ).<sup>10,11</sup> The *BCL6* protein mediates the specific binding of several transcription factors to DNA. It also may be involved in induction of germinal-center-associated functions, as it is expressed in germinal center B cells but not in plasma cells. Therefore, downregulation of *BCL6* may be necessary for terminal differentiation of B cells to memory B cells and plasma cells.<sup>12</sup>

Approximately 30 percent of DLBCLs possess a t(14;18) translocation involving the Ig heavy-chain gene and *BCL2*. Such *BCL2* gene rearrangements occur in DLBCL in either of two circumstances: (1) in

\*This chapter contains elements from the chapter written by Michael Boyiadzis and Kenneth A. Foon in the eighth edition of *Williams Hematology*.

**TABLE 9-1.** Diffuse Large B-Cell Lymphoma: Variants and Subtypes<sup>2</sup>

- I. Diffuse large B-cell lymphoma, NOS
  - A. Germinal center B-cell type
  - B. Activated B-cell type
- II. Related mature B-cell neoplasms
  - A. T-cell/histiocyte-rich large B-cell lymphoma
  - B. Primary DLBCL of the CNS
  - C. Primary cutaneous DLBCL, leg type\*
  - D. EBV-positive DLBCL, NOS
  - E. EBV-positive mucocutaneous ulcer
  - F. DLBCL associated with chronic inflammation
  - G. Lymphomatoid granulomatosis
  - H. Primary mediastinal (thymic) large B-cell lymphoma<sup>15</sup>
  - I. Intravascular large B-cell lymphoma
  - J. ALK-positive DLBCL
  - K. Plasmablastic lymphoma
  - L. Primary effusion lymphoma
  - M. HHV8+ DLBCL, NOS
  - N. High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements
  - O. High-grade B-cell lymphoma, NOS
- III. Borderline cases
  - A. B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

ALK, anaplastic lymphoma kinase; BCL, B-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; HHV, human herpes virus; NOS, not otherwise specified.

\*These represent provisional entities or provisional subtypes of other neoplasms.

DLBCLs arising by histologic transformation of a previous follicular lymphoma or (2) in *de novo* DLBCLs with a germinal center gene-expression profile. The presence of a *p53* mutation in combination with *BCL2* denotes that the tumor is derived from a histologic transformation of a prior follicular lymphoma.<sup>13</sup>

Normally, mutations in the variable region of the Ig genes confer antibody diversity in germinal center B cells. However, aberrant somatic

hypermutation occurs in more than 50 percent of cases of DLBCL. Such alterations target multiple loci, including the protooncogenes *PIM1*, *MYC*, *RhoH/TTF (ARHH)*, and *PAX5*.<sup>11</sup> The *c-MYC* gene rearrangement occurs in 10 percent of patients with DLBCL.

Gene-expression profiling studies have distinguished three molecular subtypes of DLBCL, known as (1) germinal center B-cell-like (GCB), (2) activated B-cell-like (ABC), and (3) primary mediastinal B-cell lymphoma (PMBCL).<sup>14-17</sup> GCB DLBCLs are believed to arise from normal germinal center B cells, whereas ABC DLBCLs appear to arise from postgerminal center B cells that are arrested during plasmacytic differentiation, and PMBCLs arise from thymic B cells. These DLBCL subtypes arise by distinct pathogenetic mechanisms, as judged by high-resolution, genome-wide copy-number analysis coupled with gene-expression profiling.<sup>18</sup> Furthermore, genomic studies employing massively parallel sequencing (genome/exome/RNAseq) have demonstrated common recurrent mutations in histone modification and chromatin-remodeling genes in the GCB DLBCL subtype of DLBCL, whereas mutations affecting the B-cell signaling pathway and nuclear factor (NF)  $\kappa$ B family are typical of the ABC subtype of lymphoma, as summarized in Table 9-2.<sup>19-23</sup> In addition, GCB DLBCLs often exhibit amplification of the oncogenic microRNA (miRNA)-17-92 cluster and deletion of the tumor-suppressor *PTEN*, whereas these events are rare in the ABC type of DLBCL.

## CLINICAL FEATURES

### SIGNS AND SYMPTOMS

Patients with DLBCL typically present with rapidly enlarging, symptomatic, lymphatic masses, typically in the neck or abdomen. B symptoms (drenching night sweats, fever, weight loss) are observed in approximately 30 percent of patients. Extranodal disease occurs in approximately 40 percent of patients, most commonly involving the gastrointestinal tract or marrow.<sup>24,25</sup> Other sites that may be affected include the testis, bone, thyroid, salivary glands, skin, liver, breast, nasal cavity, paranasal sinuses, and CNS. DLBCL may cause local compression of vessels (e.g., superior vena cava syndrome) or airways (e.g., tracheobronchial compression), requiring urgent treatment.

Unusual symptoms and presentations occur with some subtypes of DLBCL, such as intravascular large B-cell lymphoma, which may present with unexplained fever, or primary effusion lymphoma, which may present in immunocompromised hosts with human herpesvirus 8 infection. Approximately 60 percent of patients present with disseminated

**TABLE 9-2.** Diffuse Large B-Cell Lymphoma Subtypes Are Distinguished by Distinct Mutations in the Cells of Origin<sup>19-23</sup>

| GCB DLBCL             |           |                        | ABC DLBCL         |           |                                   |
|-----------------------|-----------|------------------------|-------------------|-----------|-----------------------------------|
| Mutation              | Frequency | Effect                 | Mutation          | Frequency | Effect                            |
| BCL2 translocation    | 25%       | Antiapoptotic          | PRDM1             | 50%       | Differentiation block             |
| EZH2 mutations        | 22%       | Histone modification   | A20 loss          | 20%       | NF- $\kappa$ B activation         |
| MEF2B mutations       | 22%       | Chromatin remodeling   | CD79B mutations   | 21%       | NF- $\kappa$ B/BCR signaling      |
| MYC translocation     | 5%        | Proliferation          | CARD 11 mutations | 11%       | NF- $\kappa$ B activation         |
| TNFRSF14 mutations    | 13%       | Immune escape          | MYD88 mutations   | 29%       | NF- $\kappa$ B/JAK-STAT signaling |
| GNA 12 & 13 mutations | 29%       | GTPases; B-cell homing |                   |           |                                   |

ABC, activated B-cell-like; BCR, breakpoint cluster region; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell-like; GTPase, guanosine triphosphatase; JAK, Janus kinase; NF- $\kappa$ B, nuclear factor kappa B; STAT, signal transducer and activator of transcription.

DLBCL (stage III or IV). Marrow involvement occurs in approximately 15 percent of patients. Discordant disease in which the lymph nodes are involved with DLBCL but the marrow contains an indolent lymphoma may occur. This combination is not associated with a poorer prognosis but increases the risk of late relapse. CNS dissemination occurs more frequently in patients with multiple extranodal sites of disease, particularly testicular, paranasal sinus, or marrow involvement<sup>26</sup> and in patients with marked elevations of serum lactic dehydrogenase (LDH). Patients at high risk for CNS involvement should undergo an examination of spinal fluid by flow cytometry for clonal B cells, which is the most sensitive method for detection of CNS disease. Patients with involvement of the Waldeyer ring have an increased risk of gastrointestinal lymphoma.

## LABORATORY FEATURES

### BLOOD AND MARROW

Lymphomatous involvement of the marrow occurs in approximately 10 to 20 percent of cases of DLBCL, with blood involvement noted on morphologic examination of blood films in approximately 3 to 8 percent of cases. These percentages are undoubtedly underestimates, and data using more sensitive tests, such as flow cytometry, are needed. Marrow involvement may lead to anemia, and in severe cases to leukopenia and thrombocytopenia, which may worsen when cytotoxic therapy is administered.

### CELL IMMUNOPHENOTYPE

The malignant cells of DLBCL express monoclonal surface Ig with  $\kappa$  or  $\lambda$  light-chain restriction. IgM is the most commonly expressed isotype of surface Ig, although occasionally cells may be negative for surface Ig.<sup>27</sup> The lymphoma cells generally express the pan-B-cell antigens CD19, CD20, CD22, PAX5, and CD79a, as well as the pan-hematopoietic antigen CD45 and, less commonly, CD10 or CD5.<sup>27,28</sup> CD5-expressing DLBCLs appear to be more aggressive and have a worse prognosis.<sup>29</sup> CD10+ DLBCL may be difficult to distinguish from Burkitt lymphoma or from grade 3 follicular lymphoma.<sup>30</sup> When a mature CD10+ B-cell phenotype is identified by flow cytometry, distinction between these possibilities should be further evaluated by morphology and genetic studies.<sup>31</sup>

### HISTOPATHOLOGY

Lymph nodes affected by DLBCL are usually effaced by a diffuse infiltrate of large lymphocytes. Three cytologic patterns are commonly recognized, namely, centroblastic, immunoblastic, and anaplastic, which are distinguished based on the size of the cells, the number of nucleoli, the basophilia of the cytoplasm, and the presence of bizarre and pleomorphic nuclei. Other rare morphologic variants occur, for example, with myxoid or fibrillary appearances. Although the diffuse growth pattern of DLBCL can be distinguished on histologic sections from the nodular growth pattern of follicular lymphoma, this distinction is usually not possible in fine-needle aspirate, body fluids, blood, or marrow specimens. Furthermore, genotypic characteristics overlap between follicular lymphoma and DLBCL, with the t(14;18)(q32;q21) translocation identified in approximately 20 percent of DLBCL and approximately 85 percent of follicular lymphomas (Chap. 10). The cells in DLBCL undergo Ig variable-region gene rearrangement and are commonly somatically mutated. Furthermore, isotype switch variants may occur.<sup>32</sup>

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis of DLBCL includes nonmalignant conditions characterized by immunoblastic infiltrates (infectious mononucleosis), nonlymphoid malignancies (carcinoma), and other lymphoma subtypes,

including Hodgkin lymphoma (Chap. 8), lymphoblastic lymphoma, and Burkitt lymphoma (Chap. 13). Adequate tissue sampling is crucial at the time of initial diagnosis, and excisional biopsies are strongly preferred to small core needle biopsies. Fine-needle aspirates are inadequate for securing a definitive diagnosis of DLBCL and are strongly discouraged. The presence of a neoplastic clone should be confirmed with molecular and/or immunophenotypic studies in most cases.

## THERAPY

### GENERAL CONSIDERATIONS

DLBCL is commonly curable with combination chemotherapy regimens containing an anthracycline. The best outcomes are obtained in patients who receive full doses of chemotherapy on schedule, without dose attenuations or treatment delays. Before therapy is instituted, several factors should be evaluated, including the patient's clinical stage, symptoms, and the International Prognostic Index (IPI). In addition, response to therapy should be evaluated according to defined criteria.<sup>33</sup> Other considerations, such as the patient's age and comorbid conditions, are important before a therapeutic intervention is selected. Future trials and therapies may be precisely tailored to subgroups of DLBCL based on biologic features of the tumor, but detailed staging, recognition of important variants, and IPI score calculation presently form the cornerstone of patient assessment.

### LIMITED-STAGE, DIFFUSE, LARGE B-CELL LYMPHOMA (STAGES I AND II)

Localized disease occurs in approximately 30 percent of patients and historically was treated with radiation therapy alone.<sup>34</sup> However, the 5-year disease-free survival (DFS) with radiation therapy in stage I disease was only 50 percent, and in stage II disease was approximately 20 percent. Combining chemotherapy with radiation therapy improved the control of local disease and resulted in a lower rate of delayed dissemination.<sup>35–40</sup> The role of chemotherapy alone, and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in particular, has been studied in several randomized trials (Table 9–3).

A Southwest Oncology Group study randomly assigned 401 patients with stage I and nonbulky stage II disease to receive either eight cycles of CHOP chemotherapy or three cycles of CHOP plus involved-field radiotherapy (IFRT).<sup>41</sup> The 5-year overall and progression-free survival (PFS) rates of the patients treated with the short-course, combined modality approach were significantly better (82 and 72 percent, respectively,  $p = 0.02$ ) than outcomes among patients treated with chemotherapy alone (77 and 64 percent, respectively,  $p = 0.03$ ). Cardiac and hematologic toxicity was greater in patients treated with eight cycles of CHOP without radiation therapy. A subset analysis using modified IPI criteria showed that patients with poor risk factors had a worse overall survival (OS). However, with longer followup, failure-free survival curves overlapped at 7 years, and the OS curves overlapped at 9 years. The early treatment advantage of CHOP plus IFRT disappeared as a result of lymphoma recurrence between 5 and 10 years after therapy.<sup>42</sup>

In an Eastern Cooperative Oncology Group (ECOG) trial involving 399 patients with bulky stage I (mediastinal or retroperitoneal mass or a mass >10 cm), stage IE, stage II, or stage IIE disease, patients who achieved complete remission (CR) after CHOP chemotherapy were randomized to observation or 30-Gy IFRT.<sup>43</sup> All patients with partial remission (PR) received 40 Gy to the involved field and radiation to the contiguous noninvolved regions. Among 172 CR patients, the 6-year DFS was 73 percent for low-dose IFRT versus 56 percent for CHOP followed by observation only ( $p = 0.05$ ) without a survival



**TABLE 9-3.** Treatment of Limited-Stage Aggressive Lymphoma

| Patient Population                              | Number of Patients | Treatment                   | 5-Year OS (p value) (%) | Ref.   |
|-------------------------------------------------|--------------------|-----------------------------|-------------------------|--------|
| Stages I and II, nonbulky                       | 401                | 8 cycles CHOP               | 72                      | 41, 42 |
|                                                 |                    | vs.<br>3 cycles CHOP + IFRT | 82<br>( $p = 0.05$ )    |        |
| Bulky stages I, IE, II, and IIE                 | 399                | 8 cycles CHOP               | 73*                     | 43     |
|                                                 |                    | vs.<br>8 cycles CHOP + IFRT | 87<br>( $p = 0.24$ )    |        |
| Age >60 years, IPI 0                            | 576                | 4 cycles CHOP               | 72                      | 44     |
|                                                 |                    | vs.<br>4 cycles CHOP + IFRT | 68<br>( $p = 0.5$ )     |        |
| Age <61 years, localized stages I and II, IPI 0 | 647                | ACVBP                       | 90                      | 45     |
|                                                 |                    | vs.<br>3 cycles CHOP + IFRT | 87<br>( $p < 0.001$ )   |        |
| Stages I and II with IPI >0                     | 60                 | R-CHOP + IFRT               | 92                      | 46     |

CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; IFRT, involved-field radiation therapy; IPI, International Prognostic Index; OS, overall survival; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.

\*OS for 172 complete remission patients randomized to observation versus IFRT.

difference between the two arms. After 6 years, failure-free survival was 63 percent in patients in PR, and conversion to CR with radiation therapy did not influence outcome. For patients in CR after eight cycles of CHOP, low-dose IFRT prolonged DFS and provided local control but did not influence survival.

In a Groupe d'Etude des Lymphomes de l'Adulte (GELA) study, 576 patients older than 60 years with stages I and II disease and an IPI score of 0 were randomized to four cycles of CHOP with or without IFRT (40 Gy).<sup>44</sup> The 5-year event-free survival (EFS) was 61 percent for patients treated with CHOP compared to 64 percent for patients treated with CHOP plus IFRT, and the OS was 72 and 68 percent, respectively. In another GELA study, 647 patients younger than age 61 years with low-risk localized aggressive lymphoma were randomized to three cycles of CHOP followed by 30 to 40 Gy of IFRT or doxorubicin (Adriamycin), cyclophosphamide, vindesine, bleomycin, and prednisone (ACVBP) chemotherapy followed by consolidation chemotherapy with methotrexate, ifosfamide, etoposide, and cytarabine.<sup>45</sup> The EFS (82 vs. 74 percent,  $p = 0.001$ ) and OS (90 vs. 87 percent,  $p < 0.001$ ) were significantly better for patients given intensive chemotherapy alone compared to patients given CHOP chemotherapy plus radiation therapy. The ACVBP regimen

cannot be given in the United States, however, because vindesine is not available.

These studies were conducted before the incorporation of rituximab into frontline regimens for DLBCL. Subsequently, in a phase II study, 60 patients older than age 60 years with at least one adverse risk factor as defined by the IPI (but excluding bulky stage II disease) received rituximab and CHOP (R-CHOP), followed by 40- to 46-Gy IFRT.<sup>46</sup> PFS was 95 percent at 2 years and 88 percent at 4 years, and OS was 95 percent at 2 years and 92 percent at 4 years. PFS and OS for historical controls not treated with rituximab were 78 and 88 percent at 4 years, respectively. Additional, retrospective, analyses suggested a role for radiotherapy in unselected DLBCL patients (including limited-stage disease) treated with R-CHOP<sup>47</sup> and in subsets age 61 to 80 years with bulky disease (7.5 cm or greater).<sup>48</sup>

Although the data available are not clear cut, at this time either three cycles of R-CHOP followed by 40 to 46 Gy of IFRT or six cycles of R-CHOP without radiotherapy represents standard therapeutic options.

## ADVANCED-STAGE, DIFFUSE, LARGE B-CELL LYMPHOMA

Combination chemotherapy (mechlorethamine, vincristine [Oncovin], procarbazine, prednisone [MOPP]) proved so successful for treatment of Hodgkin lymphoma that similar regimens were soon explored for treatment of DLBCL, including cyclophosphamide, vincristine, procarbazine, and prednisone (C-MOPP; synonym: COPP)<sup>49</sup> and cyclophosphamide 750 mg/m<sup>2</sup> IV, doxorubicin 50 mg/m<sup>2</sup> IV, vincristine 1.4 mg/m<sup>2</sup> and prednisone 100 mg orally administered daily for days 1 through 5 of each cycle (CHOP). Between 1972 and 1975, reports of CRs with long-lasting PFS with these regimens heralded the advent of curative therapy for patients with DLBCL, with CHOP administered every 21 days for six to eight cycles emerging as the most popular regimen in the United States for treatment of DLBCL (Table 9-4).

In an effort to improve upon the efficacy of CHOP, a number of intensified, multidrug combinations were developed. Early single-institution, single-armed trials showed promising results, including CR rates up to 80 percent and prolonged DFS rates of 60 percent.<sup>50,51</sup> Phase II trials of m-BACOD (moderate-dose methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone); ProMACE (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide)/CytaBOM (cytarabine, bleomycin, vincristine, methotrexate); and MACOP-B (high-dose methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin) appeared to show superior response rates compared to previously published results achieved with CHOP.<sup>52</sup> However, the benefits demonstrated in single-institution studies could not be replicated in multiinstitutional studies or with extended followup. A prospective randomized study that compared m-BACOD with CHOP showed no difference in the CR rates, DFS, or OS.<sup>53</sup> Because of these conflicting data, a four-arm phase III study was conducted and enrolled patients in a randomized prospective trial comparing CHOP, m-BACOD, MACOP-B, and ProMACE/CytaBOM.<sup>54</sup> This landmark trial enrolled 897 patients with intermediate- or high-grade lymphoma, of whom 85 percent had diffuse or follicular large cell lymphoma. In the trial, each of these regimens produced equivalent results. The DFS was 35 to 40 percent, with a 4-year survival of 36 percent in the patients who received CHOP, 34 percent in the m-BACOD group, 45 percent in those treated with ProMACE/CytaBOM, and 39 percent in the MACOP-B group ( $p = 0.14$ ). CHOP chemotherapy was the safest regimen, with only 1 percent treatment-related mortality compared to 6 percent mortality with MACOP-B. In this randomized phase III trial, the more intensive regimens offered no improvement in the remission

**TABLE 9-4.** Combination Chemotherapy for Intermediate- and High-Grade Lymphoma

| Regimen                       | Dose                       | Route               | Days of Treatment | Interval Between Treatment Cycles (Days) | Cycles |
|-------------------------------|----------------------------|---------------------|-------------------|------------------------------------------|--------|
| R-CHOP-21                     |                            |                     |                   |                                          |        |
| Rituximab                     | 375 mg/m <sup>2</sup>      | IV                  | 1                 | 21                                       | 6–8    |
| Cyclophosphamide              | 750 mg/m <sup>2</sup>      | IV                  | 1                 |                                          |        |
| Doxorubicin                   | 50 mg/m <sup>2</sup>       | IV                  | 1                 |                                          |        |
| Vincristine                   | 1.4 mg/m <sup>2</sup>      | IV                  | 1                 |                                          |        |
| Prednisone                    | 100 mg/day                 | PO                  | 1–5               |                                          |        |
| CHOP-14                       |                            |                     |                   |                                          |        |
| Cyclophosphamide              | 750 mg/m <sup>2</sup>      | IV                  | 1                 | 14                                       | 6–8    |
| Doxorubicin                   | 50 mg/m <sup>2</sup>       | IV                  | 1                 |                                          |        |
| Vincristine                   | 1.4 mg/m <sup>2</sup>      | IV                  | 1                 |                                          |        |
| Prednisone                    | 100 mg/day                 | PO                  | 1–5               |                                          |        |
| Dose-adjusted R-EPOCH*        |                            |                     |                   |                                          |        |
| Rituximab                     | 375 mg/m <sup>2</sup>      | IV                  | 1                 | 21                                       | 6–8    |
| Etoposide                     | 50 mg/m <sup>2</sup> /day  | CIV                 | 1–4 (96 hours)    |                                          |        |
| Doxorubicin                   | 10 mg/m <sup>2</sup> /day  | CIV                 | 1–4 (96 hours)    |                                          |        |
| Vincristine                   | 0.4 mg/day                 | CIV                 | 1–4 (96 hours)    |                                          |        |
| Cyclophosphamide              | 750 mg/m <sup>2</sup> /day | IV                  | 5                 |                                          |        |
| Prednisone                    | 60 mg/m <sup>2</sup> /day  | PO                  | 1–5               |                                          |        |
| ESHAP (for relapsed lymphoma) |                            |                     |                   |                                          |        |
| Etoposide                     | 40 mg/m <sup>2</sup>       | IV                  | 1–4               | 21                                       |        |
| Methylprednisone              | 500 mg/m <sup>2</sup>      | IV                  | 1–5               |                                          |        |
| Cytarabine                    | 2 mg/m <sup>2</sup>        | IV                  | 5                 |                                          |        |
| Cisplatin                     | 25 mg/m <sup>2</sup>       | CIV                 | 1–4               |                                          |        |
| DHAP (for relapsed lymphoma)  |                            |                     |                   |                                          |        |
| Dexamethasone                 | 40 mg/m <sup>2</sup>       | PO or IV            | 1–4               | 21                                       |        |
| Cisplatin                     | 100 mg/m <sup>2</sup>      | CIV                 | 1                 |                                          |        |
| Cytarabine                    | 2 gm/m <sup>2</sup>        | IV q 12 h × 2 doses | 2                 |                                          |        |
| R±ICE (for relapsed lymphoma) |                            |                     |                   |                                          |        |
| Rituximab                     | 375 mg/m <sup>2</sup>      | IV                  | 1                 | 14                                       |        |
| Mesna                         | 5000 mg/m <sup>2</sup>     | IV                  | 1 (day 2)         |                                          |        |
| Carboplatin                   | AUC = 5 (maximum 800 mg)   | IV                  | 1 (day 2)         |                                          |        |
| Etoposide                     | 100 mg/m <sup>2</sup>      | IV                  | 1–3               |                                          |        |
| Neulasta                      | 6 mg                       | SQ                  | 1 (day 4)         |                                          |        |

AUC, area under the curve; CIV, continuous intravenous infusion; I-CHOP, intensified-CHOP; SQ, subcutaneously.

The reader is advised to verify drugs, doses, and administration schedules of these regimens.

\*Doses of etoposide, doxorubicin, and cyclophosphamide are increased 20% over the dose in the previous cycle if the nadir of the absolute neutrophil count in the previous cycle was  $\geq 0.5 \times 10^9/\text{L}$ .

rate, DFS, or OS compared to the simpler and safer CHOP regimen. In retrospect, the improved complete response rates observed in the initial, single-institution phase II clinical trials of the augmented regimens appear to have been a result of enrollment of a disproportionate number of patients with favorable IPI scores.

In 2002, a major randomized clinical trial conducted by GELA demonstrated not only increased efficacy, but also minimal added toxicity with the addition of the monoclonal antibody rituximab to CHOP in older adults with DLBCL.<sup>55,56</sup> In this study, 399 patients 60 to 80 years of age with newly diagnosed DLBCL were randomized to receive either

eight cycles of CHOP given every 21 days (CHOP-21) or the same chemotherapy with eight infusions of rituximab. The combination of CHOP and rituximab significantly improved the CR rate from 63 to 76 percent, EFS from 38 to 57 percent, and OS from 57 to 70 percent compared to CHOP-21 without rituximab. There were no differences in toxicity other than a higher risk of minor cardiac events, many of which were attributed to rituximab infusion reactions. These results were confirmed by an ECOG group trial, in which 632 older patients were treated with six to eight cycles of CHOP-21 and randomized to the same chemotherapy plus five infusions of rituximab.<sup>57</sup> In this study, 2-year failure-free survival was 53 percent after a median followup of 3.5 years in patients receiving R-CHOP compared to 46 percent for patients receiving CHOP alone. A second randomization in this trial suggested that patients who received R-CHOP did not benefit from maintenance rituximab therapy. In the RICOVER-60 study, 1222 elderly patients were randomized to six or eight cycles of CHOP given every 14 days with leukocyte growth factor support (CHOP-14) or six to eight cycles of R-CHOP-14.<sup>58</sup> Six cycles of R-CHOP-14 significantly improved EFS, PFS, and OS compared to six cycles of CHOP-14. No benefit was conferred by administering eight cycles of R-CHOP-14 rather than six cycles of R-CHOP-14.

The benefit of adding rituximab to CHOP-like chemotherapy in younger patients was subsequently confirmed by the Monoclonal Antibody Therapeutic International Trial (MINT) Group.<sup>59</sup> A total of 824 patients with a good prognosis, an age-adjusted IPI of 0 or 1, and stage II to IV disease or stage I with bulky adenopathy were randomized to receive six cycles of a CHOP-like regimen or the same regimen plus rituximab. Patients with bulky (>5-cm) disease received additional radiotherapy to those areas. After a median observation time of 34 months, the addition of rituximab increased the EFS from 59 to 79 percent and the OS from 84 to 93 percent, both statistically significant findings ( $p < 0.05$ ). These results suggest that six cycles of rituximab with a CHOP-like regimen is the best therapy for young patients with good-prognosis DLBCL. Subset analysis suggested that patients with an IPI of 0 and no bulky disease represent a very favorable subgroup, with a 3-year EFS of 89 percent, whereas patients with an age-adjusted IPI of 1 and bulky disease represent a less-favorable subgroup with only a 74 percent 3-year EFS.

Investigators have attempted to improve the efficacy of R-CHOP by reducing the time between doses to every 14 days (R-CHOP-14), thereby increasing the “dose density.” Unfortunately, three randomized trials have shown no improvement in EFS, PFS, or OS with R-CHOP-14 compared to R-CHOP-21, and the dose-dense regimen was associated with increased hematologic toxicity.<sup>60,61</sup> On the other hand, an intensified combination chemotherapy regimen developed for younger patients by GELA, known as R-ACVBP (rituximab, doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone as induction, followed by consolidation, including methotrexate, etoposide, and cytarabine), was shown to have superior EFS (81 vs. 67 percent) and OS (92 vs. 84 percent) compared to R-CHOP-21 in a randomized trial of 379 DLBCL patients younger than 60 years with an age-adjusted IPI of 1.<sup>62</sup> However, patients experienced a substantially higher risk of hematologic toxicity and serious adverse events with R-ACVBP (42 vs. 15 percent with R-CHOP).

In a novel strategy to enhance chemotherapy efficacy, investigators at the United States National Cancer Institute developed an infusional chemotherapy regimen known as EPOCH (containing etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin). In this regimen, vincristine, etoposide, and doxorubicin are administered by continuous infusion over 96 hours, and cyclophosphamide is administered as a bolus. The regimen is based on *in vitro* data that showed that lymphoma cells exhibited less chemotherapy resistance when exposed to prolonged low concentrations of vincristine, doxorubicin, and etoposide than when exposed to brief, higher concentrations of the same drugs.<sup>63</sup> EPOCH

was initially evaluated in 131 patients with relapsed or refractory lymphoma and demonstrated a 74 percent overall response rate and tolerable toxicity.<sup>64</sup> Pharmacokinetics demonstrated considerable interpatient variability and suggested the need for dose adjustments to optimize outcomes for individual patients.<sup>65</sup> This observation led to incorporation of a dose-adjustment strategy based on the observed hematopoietic nadir with each cycle of treatment.<sup>66</sup> Fifty patients with previously untreated DLBCL were treated with dose-adjusted EPOCH (da-EPOCH) and demonstrated a complete response rate of 92 percent with PFS and OS rates of 70 and 73 percent, respectively. In a subsequent trial, 72 patients with untreated DLBCL were treated with da-EPOCH and rituximab with 5-year PFS and OS of 79 and 80 percent, respectively.<sup>67</sup> However, preliminary data from a randomized study comparing rituximab plus EPOCH (R-EPOCH) to R-CHOP in untreated DLBCL showed no difference in outcomes (Cancer and Leukemia Group B study 50303).<sup>68</sup> Whether da-EPOCH with rituximab improves survival in certain subsets of aggressive mature B-cell lymphomas, including high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements, remains to be proven by prospective studies.

In summary, R-CHOP administered every 21 days has emerged as the modern standard of care for DLBCL, based on the demonstration of superior outcomes and acceptable toxicity compared to CHOP in randomized clinical trials. Neither intensifying therapy by adding chemotherapy agents nor altering dose density has substantially improved upon the therapeutic ratio of the standard R-CHOP regimen. As a novel approach, infusional chemotherapy with dose-adjusted R-EPOCH may have a role for high-risk B-cell lymphoma subtypes. An improved understanding of the heterogeneous biology of DLBCL, and identification of tumor-defined prognostic subgroups with therapy modified accordingly, may soon permit tailored treatment approaches to maximize cure rates for the ABC and GCB subtypes.

## ROLE OF HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN INITIAL THERAPY

High-dose chemotherapy with autologous stem cell transplantation (ASCT) has been established as the standard of care for patients with relapsed, chemotherapy-sensitive aggressive lymphomas. However, the role of ASCT as part of initial treatment remains very contentious.<sup>69-76</sup> A recent phase III U.S. cooperative group study evaluated this question by enrolling 397 patients with high-intermediate and high-risk aggressive non-Hodgkin lymphoma who were treated with CHOP or R-CHOP and then randomized responders ( $N = 253$ ) to ASCT or further chemotherapy alone.<sup>75</sup> The ASCT group achieved superior PFS after 2 years (69 vs. 55 percent for further chemotherapy,  $p = 0.005$ ), but no difference in OS was observed (74 vs. 71 percent,  $p = 0.3$ ). In an unplanned subgroup analysis, patients with high-risk DLBCL based on the IPI achieved superior PFS and OS with ASCT.

A meta-analysis evaluated the role of high-dose chemotherapy with ASCT as part of initial treatment.<sup>77</sup> Fifteen randomized control trials including 3079 patients were eligible for this meta-analysis. Overall treatment-related mortality was 6 percent in the ASCT group, which was not significantly different than with conventional chemotherapy. Thirteen studies including 2018 patients showed significantly higher CR rates in the group receiving ASCT ( $p = 0.004$ ). However, ASCT did not have an effect on OS when compared to conventional chemotherapy. Subgroup analysis of prognostic groups according to IPI did not show any survival difference between ASCT and chemotherapy alone in 12 trials. EFS also showed no significant difference between ASCT and conventional chemotherapy.



High-dose chemotherapy and ASCT are not routinely recommended as part of frontline therapy of DLBCL. Some authorities believe that high-risk DLBCL patients who achieve at least a PR with first-line therapy may achieve superior outcomes if consolidated with ASCT, but available data supporting this approach are flawed, preventing uniform adoption. Because abbreviated courses of chemotherapy prior to transplantation impair outcomes, patients should receive a full course of standard chemotherapy even if subsequent ASCT is planned.

## RECURRENT AND REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA

### Chemotherapy

Despite major advances in initial treatment of advanced DLBCL, a substantial proportion of patients is either refractory to initial induction chemotherapy or relapses after chemotherapy. Relapse usually occurs within the first 2 years after diagnosis, and the probability of long-term survival among immunochemotherapy-treated patients who survive 2 years from DLBCL diagnosis without an event (relapse, retreatment, or death) is similar to age-matched controls.<sup>78</sup> Several second-line regimens have been evaluated in refractory and relapsed DLBCL, with response rates of 50 to 70 percent, but none of these regimens has distinguished itself as the preferred regimen. The use of single agents, such as etoposide,<sup>79</sup> cytarabine,<sup>80</sup> mitoxantrone,<sup>81</sup> lenalidomide,<sup>82</sup> and paclitaxel,<sup>83</sup> results in response rates from 20 to 40 percent; however, responses to monotherapy are generally not long lasting.

A prospective phase II study of EPOCH was examined in 131 patients with relapsed or resistant lymphoma.<sup>64</sup> In 125 assessable patients, 29 (23 percent) achieved complete responses, and 60 (48 percent) achieved partial responses. Among 42 patients with resistant disease, 57 percent responded, and in 28 patients with relapsed lymphomas, 89 percent responded with 54 percent complete responses. With a median followup of 76 months, the OS and EFS were 17.5 and 7 months, respectively. In 33 patients with chemotherapy-sensitive, aggressive disease who did not receive ASCT, EFS was 19 percent at 36 months.

The addition of rituximab to the ifosfamide-carboplatin-etoposide (ICE) chemotherapy regimen (R-ICE) increased the CR rate of patients with relapsed or primary refractory DLBCL under consideration for ASCT.<sup>84</sup> The CR rate was 53 percent, significantly better than the 27 percent CR rate ( $p = 0.01$ ) achieved among 147 similar, consecutive historical control patients with DLBCL treated with ICE. PFS for patients who underwent transplantation after R-ICE was marginally better than those of 95 consecutive historical control patients who underwent transplantation after ICE (54 vs. 43 percent,  $p = 0.25$ ).

A prospective study of 122 patients with relapsed and refractory DLBCL evaluated the role of etoposide, methylprednisolone, cytarabine, and cisplatin (ESHAP).<sup>85</sup> Forty-five patients (37 percent) attained a CR and 33 (27 percent) attained a PR, for a total response rate of 64 percent. The median duration of CR was 20 months, with 28 percent in CR at 3 years. The overall median survival duration was 14 months, with an OS rate of 31 percent after 3 years. Only 10 percent of all patients were alive and disease free after 40 months, however.

A randomized trial of second-line chemotherapy was published in 2010 comparing three cycles of either R-ICE or R-DHAP (rituximab, dexamethasone, high-dose cytarabine, and cisplatin) before planned ASCT.<sup>86</sup> This study of 396 patients demonstrated no difference in terms of efficacy (response rate 64 vs. 63 percent) or survival (3-year OS 47 vs. 51 percent) with R-ICE or DHAP, respectively. In the absence of high-level evidence supporting one salvage regimen over another, treatment for relapsed/refractory DLBCL requires individualized consideration of comorbidities and patient factors.

### Autologous Stem Cell Transplantation

The role of ASCT in relapsed DLBCL was demonstrated in a randomized trial of 109 patients who responded to salvage chemotherapy with the DHAP chemotherapy regimen and were randomly assigned to receive four courses of chemotherapy plus radiotherapy (54 patients) or radiotherapy plus intensive chemotherapy and ASCT (55 patients).<sup>87</sup> After 5 years, the EFS was 46 percent in the transplantation group and 12 percent in the chemotherapy/radiotherapy group ( $p = 0.001$ ), and the rate of OS was 53 and 32 percent, respectively ( $p = 0.038$ ). Patients with relapsed or primary refractory DLBCL who achieved CR before ASCT had better outcomes than those who achieved only PR. Disease sensitivity at the time of ASCT and time from initial diagnosis to relapse remain key prognostic variables for predicting treatment outcome after standard ASCT.<sup>86,87</sup> Patients who undergo ASCT when the disease is resistant to the initial induction therapy have less than a 20 percent probability of durable DFS.

### Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has also been used in patients with DLBCL. The European Bone Marrow Transplant Group performed a case-controlled study by matching 101 allo-HSCT patients with 101 ASCT patients.<sup>88</sup> The PFS was similar in both types of transplants (49 percent for allo-HSCT vs. 46 percent for ASCT). The overall relapse and progression rate for the allo-HSCT patients was 23 percent compared with 38 percent for the ASCT patients. This difference was not statistically significant. Nine patients who had undergone ASCT died from early procedure-related toxicity, and 17 patients who had undergone allo-HSCT died from early procedure-related toxicity. To reduce the treatment-related mortality associated with allo-HSCT, non-myeloablative preparative regimens were developed to minimize the toxicity associated with standard high-dose chemotherapy, achieve sufficient engraftment to prevent graft rejection, and exploit the graft-versus-tumor effect of allo-HSCT. In a prospective study, 31 patients with DLBCL and one patient with Burkitt lymphoma received allo-HSCT following 2-Gy total-body irradiation with or without fludarabine.<sup>89</sup> Twenty-four patients had undergone prior ASCT. With a median followup of 45 months, 3-year OS and PFS were 45 and 35 percent, respectively. Three-year cumulative incidences of relapse and nonrelapse mortality were 41 and 25 percent, respectively. Cumulative incidences of acute graft-versus-host disease (GVHD) grades II to IV, grades III and IV, and chronic GVHD were 53, 19, and 47 percent, respectively. In another study, 48 consecutive patients with relapsed or refractory DLBCL (30 patients with *de novo* disease and 18 patients with transformed follicular lymphoma) underwent transplantation with an alemtuzumab-containing regimen.<sup>90</sup> The PFS and OS rates after 4 years were 48 and 47 percent, respectively. Seventeen percent of patients developed grades II to IV acute GVHD, and 13 percent experienced extensive chronic GVHD. Four-year estimated nonrelapse mortality was 32 percent, and relapse risk was 33 percent. Although these results are promising, allo-HSCT cannot be recommended before ASCT except in the context of a clinical trial.

### Radioimmunotherapy

Radioimmunotherapy as monotherapy is not recommended for DLBCL, but its role as part of a conditioning regimen prior to ASCT has been encouraging. A phase II trial evaluated the safety and efficacy of combining <sup>90</sup>Y-ibritumomab tiuxetan with high-dose carmustine (bischloroethylnitrosourea), cytarabine (Ara C), etoposide, and melphalan (BEAM) and ASCT in patients with lymphoma who were considered ineligible for total-body irradiation because of older age or prior radiotherapy.<sup>91</sup> The addition of <sup>90</sup>Y-ibritumomab tiuxetan to BEAM with ASCT was feasible and the toxicity and tolerability profile similar to that observed with BEAM alone. Similarly, <sup>131</sup>I-tositumomab (up to 0.75 Gy) was combined with BEAM followed by ASCT for the treatment of chemotherapy-resistant

relapsed or refractory lymphoma. Short-term and long-term toxicities were similar to that in patients previously treated with BEAM alone, with an OS rate of 55 percent and an EFS rate of 39 percent.<sup>92</sup>

### Summary of Approach to Patients with Relapsed Disease

Patients with relapsed disease should receive multidrug chemotherapy, such as R-ICE or R-DHAP. If chemosensitivity is demonstrated and no contraindications are present, ASCT should be performed. If patients are elderly or have comorbid conditions, the goal should be palliation. Radiotherapy can be used to alleviate symptoms at particular sites of involvement in patients with relapsed DLBCL, and single-agent therapy can be used but with low expected response rates and duration of responses.

## COURSE AND PROGNOSIS

### INTERNATIONAL PROGNOSTIC INDEX

In 1993, a model was proposed to assign a prognosis to patients with aggressive lymphoma undergoing treatment with doxorubicin-containing chemotherapeutic regimens; the model was termed the *international prognostic index* (IPI).<sup>93,94</sup> The model used clinical data, including (1) tumor stage, (2) serum LDH level, (3) number of extranodal disease sites involved, (4) performance status, and (5) patient age. This model resulted in the IPI, which is used to forecast the behavior of aggressive lymphoma (Table 9-5 and Fig. 9-1). For patients younger than age 60 years, an age-adjusted IPI has been proposed in which all the factors of the IPI are used, except for age and presence of extranodal sites. The 5-year survival rates for patients age 60 years or younger with IPI scores of 0, 1, 2, and 3 were 83, 69, 46, and 32 percent, respectively (Table 9-6).<sup>93</sup> To better estimate prognosis among modern DLBCL patients, a revision of the IPI was recently developed by the National Comprehensive Cancer Network (the NCCN-IPI).<sup>95</sup> This model employs the same five risk factors but uses a different scoring algorithm and improves discrimination of groups treated with rituximab-containing chemoimmunotherapy.

## GENE-EXPRESSION PROFILING AND DETERMINATION OF DIFFUSE LARGE B-CELL LYMPHOMA SUBTYPES

Gene-expression profiling has also been used to delineate groups of patients with DLBCL who may differ in their response to therapy and prognosis (Fig. 9-2).<sup>14-17,96,97</sup> Six genes identified by gene-expression analysis and detected by quantitative real-time polymerase chain reaction can

identify three prognostic groups in patients with DLBCL.<sup>98</sup> The six genes that were used in this model occur in the germinal center B-cell signature (*LMO2*, *BCL6*), activated B-cell signature (*BCL2*, *CCND2*, *SCYA3*), and lymph node signature (*FN1*). In this study, expression of *LMO2*, *BCL6*, and *FN1* correlated with prolonged survival, whereas expression of *BCL2*, *CCND2*, and *SCYA3* correlated with short survival. Protein immunohistochemistry (IHC) has also been used to delineate DLBCL subtypes, but shows imperfect concordance with gene-expression results in determining cell of origin.<sup>96</sup> Nonetheless, IHC assessment of cell of origin is employed to categorize DLBCL in the modern WHO classification.<sup>2</sup> Results of ongoing prospective trials may lead to new first-line regimens for DLBCL not otherwise specified (NOS) based on cell of origin, but initial efforts to overcome the adverse prognosis of the activated B-cell type of DLBCL NOS have been unsuccessful.<sup>98a,98b</sup>

### SERUM LACTATE DEHYDROGENASE AND $\beta_2$ -MICROGLOBULIN

Patients with an elevated  $\beta_2$ -microglobulin level and high serum LDH have a poor prognosis, with a 26 percent survival compared to 81 percent survival in patients without elevation of either of these markers.<sup>93,99</sup>

### PRESENCE OF MYC GENE REARRANGEMENT OR ELEVATED PROTEIN EXPRESSION

Approximately 10 percent of DLBCL patients harbor a translocation involving the *MYC* gene. Patients with an *MYC* rearrangement detected by fluorescence *in situ* hybridization (FISH) have been shown to have an inferior 5-year survival of 33 percent when treated with standard R-CHOP therapy, compared to 72 percent survival for those without *MYC* rearrangements.<sup>100</sup> Concurrent translocation of *BCL2* or *BCL6* is seen in a subset of cases. These “double-hit” lymphomas are classified as “high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements” except for cases that fulfill morphologic criteria for a follicular or lymphoblastic lymphoma.<sup>2</sup> *MYC* protein overexpression by IHC is also associated with an inferior prognosis, particularly when accompanied by *BCL2* protein overexpression.<sup>101,102</sup>

### POSITRON EMISSION TOMOGRAPHY

Fluorine-18-fluorodeoxyglucose-positron emission tomography (FDG-PET) is used for initial staging and at the end of treatment (to assess remission status) in patients with DLBCL. FDG-PET is superior to computed tomographic imaging in detecting nodal and extranodal sites of aggressive lymphoma, with the potential to alter stage, prognosis, and selection of therapy.<sup>103</sup> In addition, FDG-PET performed at the end of therapy is highly informative. A negative PET predicts a high probability of disease control and is required to designate a CR by modern response criteria.<sup>33,104</sup> Other uses of PET in DLBCL are investigational or not routinely recommended.

## PRESENTATION AND THERAPY FOR SPECIFIC DIFFUSE LARGE B-CELL LYMPHOMA SUBTYPES AND RELATED MATURE B-CELL NEOPLASMS

### PRIMARY TESTICULAR LYMPHOMA

Primary testicular lymphoma represents 1 to 2 percent of all lymphomas, with an estimated incidence of 0.26 per 100,000 males per year.<sup>105</sup> Even though lymphomas account for only 1 to 7 percent of all testicular

**TABLE 9-5.** International Prognostic Factor Index for Non-Hodgkin Lymphoma<sup>93</sup>

#### Risk factors

- Age older than 60 years
- Serum lactic dehydrogenase greater than twice normal
- Performance status  $\geq 2$
- Stage III or IV
- Extranodal involvement at more than 1 site

Each factor accounts for 1 point, for a total score that ranges from 0 to 3 for patients younger than 61 years of age, with this age-adjusted index including all variables except for age and extranodal sites. For patients 61 years of age and older, a total score ranges from 0 to 5 and includes each variable shown in this table.

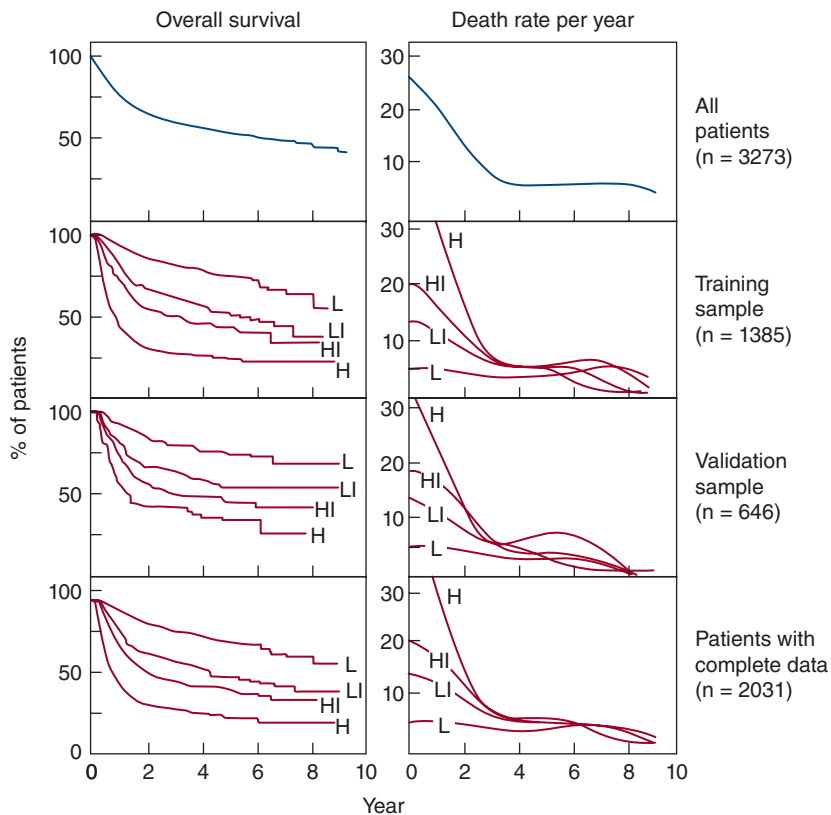


**Figure 9-1.** (Left panels) Kaplan-Meier survival curves for the four risk groups.<sup>94</sup> (Right panels) Death rates during the study period. Only 2031 of the 3273 patients had sufficient relevant information for classification according to the international index. H, high risk; HI, high-intermediate risk; L, low risk; LI, low-intermediate risk.

malignancies, they represent the most common testicular tumor in men older than 50 years of age. Histologically, 80 to 90 percent of primary testicular lymphomas are DLBCL; individuals have a mean age at diagnosis of 68 years (range: 21 to 98 years).<sup>106,107</sup> Most patients present with stage I or II disease with isolated involvement of the right or left testis equal in frequency, whereas 6 percent of cases have bilateral involvement. Primary testicular lymphoma shows a tendency to disseminate to several extranodal sites, including the contralateral testis, CNS, skin, Waldeyer ring, lung, pleura, and soft tissues. Treatment using radiation

therapy alone provides suboptimal disease control, even for patients with stage I disease. Chemotherapy without anthracyclines was shown to produce inferior results compared with regimens containing anthracyclines. Thus, R-CHOP is the regimen recommended by most lymphoma specialists following orchiectomy for testicular DLBCL, with a median OS of 4.4 years. Radiation therapy to the contralateral testis should also be administered.<sup>105,108,109</sup> CNS prophylaxis with intrathecal chemotherapy or high-dose methotrexate should be strongly considered because of the observed propensity for CNS relapses.

**TABLE 9-6.** Outcome According to Risk Group Defined by the International Prognostic Index<sup>93</sup>

| International Index                                                    | No. of Risk Factors | Complete Response | Relapse-Free Survival (%) |        | Survival (%) |        |
|------------------------------------------------------------------------|---------------------|-------------------|---------------------------|--------|--------------|--------|
|                                                                        |                     | Rate (%)          |                           |        |              |        |
| INTERNATIONAL PROGNOSTIC INDEX, ALL PATIENTS                           |                     |                   |                           |        |              |        |
|                                                                        |                     |                   | 2-Year                    | 5-Year | 2-Year       | 5-Year |
| Low                                                                    | 0 or 1              | 87                | 79                        | 70     | 84           | 73     |
| Low-intermediate                                                       | 2                   | 67                | 66                        | 50     | 66           | 51     |
| High-intermediate                                                      | 3                   | 55                | 59                        | 49     | 54           | 43     |
| High                                                                   | 4 or 5              | 44                | 58                        | 40     | 34           | 26     |
| AGE-ADJUSTED INTERNATIONAL PROGNOSTIC INDEX, PATIENTS <61 YEARS OF AGE |                     |                   |                           |        |              |        |
|                                                                        |                     |                   | 2-Year                    | 5-Year | 2-Year       | 5-Year |
| Low                                                                    | 0                   | 92                | 88                        | 86     | 90           | 83     |
| Low-intermediate                                                       | 1                   | 78                | 74                        | 66     | 79           | 69     |
| High-intermediate                                                      | 2                   | 57                | 62                        | 53     | 59           | 46     |
| High                                                                   | 3                   | 46                | 61                        | 58     | 37           | 32     |



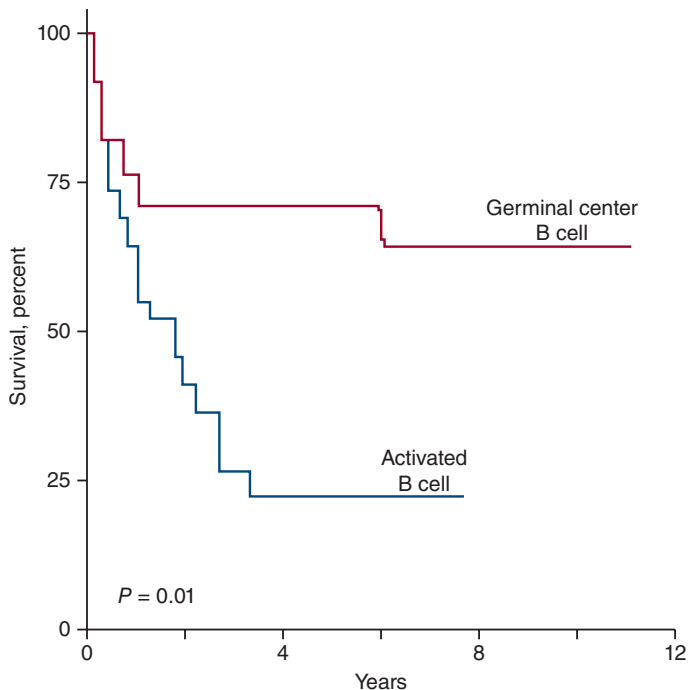


**Figure 9-2.** Overall survival in a group of patients with diffuse large B-cell lymphoma whose cell of origin was determined by gene-expression profiling.<sup>14</sup> Survival of patients with diffuse large B-cell lymphoma whose malignant cells were thought to arise from a germinal center B cell was significantly better than in patients whose cell of origin arose from activated B cells.

## LYMPHOMA DURING PREGNANCY

Lymphoma is the fourth most frequent malignancy diagnosed during pregnancy, occurring in approximately 1 in 6000 deliveries.<sup>110</sup> Reports of therapeutic interventions in pregnant patients with lymphoma are limited, and management recommendations are largely based on small retrospective studies and case reports. Both radiation therapy and chemotherapy during pregnancy are potentially teratogenic. Fetal exposure to antineoplastic agents may result in impaired growth, diminished neurologic and/or intellectual function, decreased gonadal and reproductive function, mutagenesis of germline tissue, and carcinogenesis.<sup>111</sup> The risks of treatment to the fetus are greatest during the first trimester, and therapeutic abortion is a consideration under these circumstances. CHOP during the second and third trimesters may be administered relatively safely with little risk of significant adverse fetal outcomes.<sup>112,113</sup> The prognosis of patients who receive optimal chemotherapy is similar to that of nonpregnant patients.<sup>114</sup>

Only a few cases of rituximab administration during pregnancy have been reported, most of them for the treatment of nonmalignant disorders such as autoimmune diseases. Patients with supradiaphragmatic stage I disease may be considered for localized radiotherapy as a temporary measure until the second trimester, when chemotherapy holds less risk for the fetus.<sup>115</sup> Patients in the second or third trimester should be treated with full-dose chemotherapy.

## PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA

### Definition

Primary mediastinal large B-cell lymphoma arises in the mediastinal lymphatic structures, probably from a thymic B-cell precursor.

### Epidemiology

This variant type of DLBCL accounts for approximately 3 percent of lymphomas and is most commonly seen in young and middle-aged adults, with about two-thirds of cases occurring in females.

### Clinical Features

The clinical presentation is typically with an anterior mediastinal mass that is locally invasive into neighboring tissues, including the lungs and pericardium. Upper airway obstruction and superior vena cava syndrome occur in approximately 40 percent of patients.<sup>116</sup> Regional lymph nodes, especially the cervical chain, are often involved, but distant nodal involvement at presentation is uncommon with this entity. Relapses tend to be extranodal, including sites such as the liver, gastrointestinal tract, kidneys, ovaries, and CNS. Marrow involvement is very unusual.

### Laboratory Features

PMBCL and Hodgkin lymphoma exhibit similar gene-expression profiles, raising questions about biologic relationships.<sup>16,117</sup> Sometimes bizarre multinucleated cells mimicking Reed-Sternberg cells are seen in PMBCL along with other morphologic features suggestive of Hodgkin lymphoma. Fibrotic bands may be prominent in biopsies, leading to the appellation “primary B-cell mediastinal lymphoma with sclerosis.” IHC may be helpful in the differential diagnosis since primary mediastinal lymphoma expresses weak CD30, lacks CD15 antigen as seen in Hodgkin lymphoma, and expresses B-cell-associated antigens CD19, CD20, CD22, and CD79a.<sup>118</sup> Other useful markers include the melanocytic marker, HMB-45, keratin, and placental leukocyte alkaline phosphatase, which can help distinguish PMBCL from sarcoma, melanoma, thymoma, and seminoma.

### Therapy

Several regimens have been evaluated in PMBCL. A retrospective study compared the outcomes of 426 patients who had fibrotic tumor reactions (sclerosis) and previously untreated disease using CHOP-like regimens, third-generation (MACOP-B, VACOP-B [etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin], ProMACE CytaBOM) regimens, or high-dose chemotherapy with autologous hematopoietic stem cell transplantation.<sup>119</sup> With chemotherapy, the CR rates were 49, 51, and 53 percent with first-generation, third-generation, and high-dose chemotherapy treatments, respectively. All patients who achieved CR or PR had radiation therapy to the mediastinum. The final CR rates were 61 percent for CHOP-like regimens, 79 percent for MACOP-B and other regimens, and 75 percent for high-dose chemotherapy/autologous hematopoietic stem cell transplantation. Projected 10-year PFS rates were 35, 67, and 78 percent, respectively, and projected 10-year OS rates were 44, 71, and 77 percent, respectively.

In another retrospective study of 138 patients with PMBCL, the effectiveness of two chemotherapy regimens (CHOP vs. MACOP-B/VACOP-B) and the role of mediastinal IFRT as consolidation were evaluated.<sup>120</sup> CR occurred in 51 percent of the CHOP group and 80 percent in MACOP-B/VACOP-B. EFS was 40 percent with CHOP and 76 percent in the MACOP-B/VACOP-B group. The addition of IFRT improved the outcome, regardless of the type of chemotherapy used. The incorporation of rituximab in the dose-adjusted EPOCH regimen, without radiotherapy consolidation, resulted in 97 percent OS and 93 percent EFS in a nonrandomized, phase II clinical trial.<sup>121</sup> Based on this study, which included 5-year followup data, many authorities believe that the dose-adjusted R-EPOCH regimen should be the preferred first-line regimen for this entity, without routine incorporation of radiotherapy.

## LYMPHOMATOID GRANULOMATOSIS

### Definition

Lymphomatoid granulomatosis is a rare lymphoproliferative disorder characterized by angiocentric and angiodestructive Epstein-Barr virus (EBV)-driven B-cell proliferation associated with extensive reactive T-cell infiltration.<sup>2</sup>

### Epidemiology

Approximately two-thirds of cases occur in males. The median age of presentation is in the fifth decade of life, although pediatric cases occur.

### Clinical Features

The lung is the most common site of involvement (90 percent), followed by the skin (25 to 50 percent), kidney (30 to 40 percent), liver (29 percent), and CNS (26 percent). The spleen and the lymph nodes are uncommonly involved.<sup>122</sup> Nearly all patients are symptomatic at presentation, with cough, dyspnea, chest pain, fever, weight loss, and joint pain. Abdominal pain and diarrhea occur as a result of gastrointestinal involvement, and various neurologic signs, including diplopia, ataxia, and mental status changes, may occur as a result of CNS involvement. Skin manifestations are clinically diverse and include ulcerations, plaques, and maculopapules, although subcutaneous nodules are most common. Pulmonary involvement typically involves bilateral, lower lobe nodules, which frequently cavitate. Nodules may also be found in the brain and kidney and other locations.

### Laboratory Features

**Histopathology** The grading of lymphomatoid granulomatosis depends upon the proportion of EBV-positive B cells identified by *in situ* hybridization with an EBV-encoded RNA probe relative to the number of reactive lymphocytes in the background.<sup>2</sup> Grade 1 lesions contain a polymorphous lymphoid infiltrate without cytologic atypia. Large transformed lymphoid cells are absent or rare, and EBV positivity is detectable in only a few cells. Grade 2 lesions contain occasional large lymphoid cells or immunoblasts in a polymorphous background. *In situ* hybridization for EBV readily identifies EBV-positive cells, which are present at 5 to 20 per high-power field. Grade 3 lesions continue to show an inflammatory background but contain frequent large atypical B cells that are CD20 positive. By *in situ* hybridization, EBV-positive cells are numerous (>50/high-power field).

### Therapy and Prognosis

The clinical prognosis is variable in lymphomatoid granulomatosis, with a median survival of 2 years.<sup>123</sup> Poor prognostic features include neurologic involvement and higher pathologic grade. The disease is uncommon, and the optimal treatment regimen is unclear. In a prospective study, patients with grades I and II disease were treated with interferon- $\alpha$ , whereas those with grade III lesions received dose-adjusted R-EPOCH chemotherapy.<sup>124</sup> Among 27 patients with grade I or II disease treated with interferon- $\alpha$ , 56 percent were in continuous CR for a median of 52 months. Among grade III patients treated with dose-adjusted R-EPOCH, 40 percent achieved CR, with OS and PFS rates of 69 and 82 percent, respectively, after a median followup of 46 months.

## INTRAVASCULAR LARGE B-CELL LYMPHOMA

### Definition

Intravascular large B-cell lymphoma is a rare type of extranodal large B-cell lymphoma characterized by selective growth of lymphoma cells within the lumina of vessels, sparing the large arteries and veins.<sup>2</sup>

### Epidemiology

This tumor usually occurs in adults in the sixth and seventh decade. It occurs equally in men and women.

### Clinical Features

The clinical manifestations of this lymphoma are extremely variable, with most symptoms related to the organs affected. Two major patterns of clinical presentation have been recognized. The first is in European countries, with individuals showing brain and skin involvement; the second is in Asian countries, where patients typically present with multiorgan failure, hepatosplenomegaly, pancytopenia, and hemophagocytic syndrome.<sup>125–129</sup> B symptoms (fever, drenching sweats, and weight loss) are common in both types. An isolated cutaneous variant almost unique to Western countries has been identified in females and is associated with a better prognosis.<sup>125</sup> In this variant, skin lesions range from single to striking clusters of nodules and tumors, which may appear as violaceous plaques, erythematous nodules, or ulcerating tumors, which are often painful. These lesions commonly appear on the arms and legs, abdomen, and breasts but may occur anywhere.

### Laboratory Features

There are no laboratory findings specific for intravascular B-cell lymphoma. Increased LDH and  $\beta_2$ -microglobulin levels are observed in most patients. An elevated erythrocyte sedimentation rate and abnormalities in hepatic, renal, and thyroid function are also common.<sup>130</sup> Tumor cells express B-cell-associated antigens and occasionally express CD5.

### Therapy

Anthracycline-based chemotherapy is the standard of care for this type of lymphoma, and retrospective studies suggest that the addition of rituximab improves clinical outcomes.<sup>131,132</sup> One study analyzed 106 patients who received chemotherapy either with rituximab (R-chemotherapy,  $n = 49$ ) or without rituximab (chemotherapy,  $n = 57$ ).<sup>132</sup> The CR rate was 82 percent for patients in the R-chemotherapy group compared to 51 percent in the chemotherapy-alone group ( $p = 0.001$ ). PFS and OS rates 2 years after diagnosis were 56 and 66 percent, respectively, in the R-chemotherapy group, compared with 27 and 46 percent, respectively, for patients in the chemotherapy-alone group ( $p = 0.001$  for PFS and  $p = 0.01$  for OS). More intensive chemotherapy, including high-dose methotrexate and/or high-dose cytarabine, is recommended for patients with CNS involvement (e.g., R-CHOP with high-dose methotrexate). Many authorities believe that CNS prophylaxis is warranted even in cases of intravascular large cell lymphoma without demonstrable CNS disease at diagnosis because of the high rate of CNS relapse.

## POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS

### Definition

Posttransplantation lymphoproliferative disorders (PTLDs) result from lymphoid or plasmacytic proliferations that develop in the setting of solid-organ or marrow transplantation. Although PTLD represents an uncommon complication in transplant patients, it is a significant cause of morbidity and mortality.<sup>133</sup>

### Epidemiology

The incidence of PTLD is approximately 1 to 2 percent in solid-organ transplant recipients, which is 30 to 50 times higher than the incidence of lymphoproliferative diseases in the immunocompetent general population.<sup>134</sup> There is a clear association between PTLD and the type of organ transplanted. Among the most commonly transplanted solid

organs, heart, lung, and intestinal transplantation have the highest incidences of PTLT, with the highest risk occurring in the first year after transplantation. The incidence of PTLT after blood or marrow transplantation is lower than after solid-organ transplantation and ranges from 0.5 to 1.0 percent in reported series.

### Pathogenesis

The major risk factors that have been identified for the development of PTLT include EBV-positive serology pretransplantation, type of organ transplanted, and intensity of immunosuppressive regimen used.<sup>136–138</sup> The onset of posttransplantation lymphoma in most patients is related to B-cell proliferation induced by infection with EBV in the setting of chronic immunosuppression. The genome of the virus can be detected in the cells of the majority of cases.<sup>139</sup> However, in approximately 20 to 30 percent of cases the virus is undetectable in involved tissues.<sup>140</sup> Involvement of the lymph nodes, gastrointestinal tract, lungs, and liver is common with all types of allografts. The majority of PTLTs in solid-organ transplant recipients are of host origin. In contrast, the majority of PTLTs in hematopoietic stem cell allografts are of donor origin. Involvement of the grafted organ occurs in approximately 30 percent of patients and may lead to organ damage and fatal complications.<sup>141</sup>

### Therapy

Management of PTLT is not uniform. A wide variety of approaches, including decreasing the dose of immunosuppressive drugs or administering antiviral therapy, interferon, intravenous Ig, adoptive therapy with EBV-specific cytotoxic T lymphocytes, chemotherapy, radiation, and rituximab therapy, have been reported. If feasible, reduction of immunosuppression is the first step in the management of such patients. Many cases of polyclonal PTLT resolve completely with a reduction in immunosuppressive therapy.<sup>142</sup> Patients with late PTLT and more aggressive monoclonal PTLT are less likely to respond.<sup>143</sup> Rituximab has shown promising results in the treatment of CD20+ PTLT. In a multicenter prospective trial, 43 patients with previously untreated B-cell PTLT who failed to respond to tapering of immunosuppression were treated with four weekly injections of rituximab at 375 mg/m<sup>2</sup>.<sup>144</sup> The overall response rate was 44 percent, with an OS of 86 percent after 80 days and 67 percent after 1 year. The only baseline factor predicting response at day 80 was a normal level of serum LDH. A retrospective study evaluated the efficacy and safety of chemotherapy salvage therapies in adult recipients of solid-organ transplants with a second progression of PTLT after initial therapy with rituximab.<sup>145</sup> CHOP therapy achieved a favorable overall response rate of 70 percent in this setting, indicating that PTLT generally remains chemotherapy sensitive after progression following initial therapy with rituximab.

A prospective trial evaluated a stepwise treatment approach beginning with reduction of immunosuppression, then interferon- $\alpha$ , and finally chemotherapy with ProMACE-CytaBOM plus granulocyte-monocyte colony-stimulating factor.<sup>146</sup> Sixteen eligible patients began treatment with reduced immunosuppression. The response rate to reduced immunosuppression was zero of 16 CR and one of 16 PR (6 percent). Six of the 16 patients (38 percent) had documented rejection of the transplanted organ during the period of reduced immunosuppression. Eight of the 16 patients had documented progressive disease during the period of reduced immunosuppression. Thirteen patients underwent treatment with interferon, with two patients achieving CRs (15 percent) and two achieving PRs (15 percent). Seven eligible patients proceeded to ProMACE-CytaBOM chemotherapy, with five (67 percent) attaining CR. Four of the five CRs experienced remission durations of more than 2 years. The median survival for the treatment cohort as a whole was 19 months, with a range of 5 days to 60+ months. Overall survival was 50 percent at 2 years, 44 percent at 4 years, and 24 percent at 8 years.

The following sequence is currently recommended for PTLT: The first intervention should be reduction in immunosuppression, followed by four weekly cycles of rituximab if reduction of the immunosuppression by itself is ineffective. If PTLT does not regress with these measures, then six cycles of R-CHOP are recommended.

## T-CELL/HISTIOCYTE-RICH LARGE B-CELL LYMPHOMA

### Definition

T-cell/histiocyte-rich large B-cell lymphoma is characterized by effacement of the architecture of the lymph node by a lymphohistiocytic infiltrate with a diffuse or vaguely nodular growth pattern.<sup>2,147</sup> There are a limited number of large atypical B cells occurring singly or in small clusters with a predominant background infiltrate composed of T cells and histiocytes, the latter usually of the nonepithelioid type.

### Epidemiology

T-cell/histiocyte-rich large B-cell lymphoma accounts for less than 5 percent of all cases of DLBCL and occurs at a younger age on average. The median age of onset is in the fourth decade, compared to the sixth decade for DLBCL.<sup>148–151</sup> A male predominance is noted in most series, in contrast to DLBCL not otherwise specified, which occurs equally in men and women.

### Clinical Features

This variant more often presents with advanced-stage disease, B symptoms, an elevated LDH, splenic infiltration, and multiple extranodal sites (especially the marrow and liver) compared to standard DLBCL.<sup>148–150,152</sup>

### Therapy and Prognosis

When treated with CHOP-like regimens, most series suggest that the outcome for these patients is similar to patients with typical DLBCL,<sup>149,151–154</sup> with CR rates of approximately 60 percent and 3-year and 5-year OS rates of 50 to 64 percent and 45 to 58 percent, respectively. Two case-control analyses have been performed comparing T-cell/histiocyte-rich large B-cell lymphoma and DLBCL, with no differences in OS observed.<sup>149,150</sup> Based on these data, this subtype of large B-cell lymphoma should be treated in the same fashion as traditional DLBCL. Six cycles of R-CHOP for advanced disease are a reasonable initial approach to therapy.

## PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE

### Definition

Primary cutaneous diffuse large B-cell lymphoma, leg type, is a primary cutaneous lymphoma composed solely of large transformed B cells that exhibit a predilection for the skin of the leg.<sup>2</sup>

### Epidemiology

Primary cutaneous DLBCLs of the leg type constitute approximately 4 percent of all primary cutaneous B-cell lymphomas.<sup>2,155</sup> The median age at the time of presentation is 60 to 70 years.

### Clinical Features

These lymphomatous tumors affect the skin of the legs in most cases, but approximately 10 percent arise at other sites.<sup>156–158</sup> Multiple tumors, sometimes ulcerating, are associated with poorer prognosis. There are frequent relapses, and extracutaneous dissemination may occur.



### Laboratory Features

The malignant B cells in this entity usually express CD20, BCL2, and FOXP1. FISH of the lymphoma cells often detects translocations involving *MYC*, *BCL6*, or *IGH* genes. Amplification of the *BCL2* gene is usually responsible for the high frequency of BCL2 overexpression. The t(14;18) translocation is generally not observed in this lymphoma variant. The gene-expression profile observed in this subtype suggests an activated B-cell origin.

### Therapy

Anthracycline-containing chemotherapy with rituximab should be considered as initial therapy. The incorporation of rituximab improves the response rates and OS.<sup>156–158</sup> However, because of the advanced age of most patients with this variant at the time of diagnosis, aggressive combination chemoimmunotherapy may not be feasible due to patient frailty or comorbidities. In such circumstances, local radiation therapy or less-aggressive chemotherapy is reasonable.

## ANAPLASTIC LYMPHOMA KINASE-POSITIVE LARGE B-CELL LYMPHOMA

### Definition

Anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma is an uncommon neoplasm of large immunoblast-like B cells that stain for nuclear and/or cytoplasmic ALK protein.

### Epidemiology

The average age at presentation is in the fourth decade, with a male predilection. Most patients present with advanced-stage disease.

### Clinical Features

Patients with anaplastic DLBCL usually present with widespread disease, with cervical and mediastinal nodes being the most frequent sites of adenopathy, and the liver, spleen, bone, and gastrointestinal tract being the most common sites of extranodal involvement.<sup>159,160</sup>

### Laboratory Features

The immunoblastic cells of anaplastic DLBCL typically have a large central nucleolus and may exhibit maturation to plasmablastic cells. The lymphoma cells stain for the ALK protein by immunohistochemistry, usually with a granular cytoplasmic appearance, though nuclear staining may also occur. These cells are usually negative for CD3, CD20, CD30, and CD79a. MUC1 mucin, a high-molecular-weight transmembrane glycoprotein, also known as epithelial membrane antigen, and CD138 are usually strongly expressed by these cells. Monoclonal (light-chain restricted) IgA or IgG is generally present in the cytoplasm of the malignant cells. Occasional cases possess a t(2;17)(p23;q23) translocation that results in a clathrin-ALK fusion protein.

### Therapy and Prognosis

The clinical course of ALK-positive large B-cell lymphoma is aggressive, with a median survival time of 24 months. Because the tumors are usually negative for CD20, the utility of rituximab is dubious for this entity. Anthracycline-based chemotherapy is often inadequate, and more intensive therapies should be considered.<sup>159,160</sup>

## HUMAN IMMUNODEFICIENCY-RELATED DIFFUSE LARGE B-CELL LYMPHOMA VARIANTS

Primary effusion lymphoma, plasmablastic lymphoma, and large B-cell lymphoma arising in human herpesvirus-8-associated multicentric

Castleman disease usually develop in the setting of acquired immunodeficiency induced by HIV.

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## CHAPTER 10

# FOLLICULAR LYMPHOMA

Oliver W. Press and John P. Leonard

### SUMMARY

Follicular lymphoma (FL) is an indolent, neoplastic disorder of germinal center-derived B lymphocytes that affects approximately 14,000 people in the United States each year. It typically presents as a disseminated disorder with painless, diffuse lymphadenopathy and marrow infiltration and may be associated with hepatosplenomegaly and circulating lymphoma cells in the blood. A characteristic translocation, t(14;18), is found in the cells of 85 percent of patients, which deregulates BCL2 protein expression and inhibits apoptosis of affected B cells. The cells typically express monoclonal surface immunoglobulin, CD10, CD19, CD20, CD22, CD45, and CD79a on their cell surface, but not CD5 or CD23. Patients are often asymptomatic at the time of presentation and may live for many years in good health without therapy. Most patients die with disease rather than from disease. On the other hand, it is expected that most patients eventually develop progressive lymphadenopathy, causing symptoms mandating intervention. Many treatment regimens are effective at inducing remissions, including single-agent rituximab or chlorambucil, or there are several multidrug programs, including bendamustine plus rituximab (BR); rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP); and rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). None of these therapies, however, is considered curative, and most patients eventually relapse with recurrent disease. Autologous and allogeneic hematopoietic cell transplantation (HCT) can induce prolonged remissions in many patients with relapsed FL, but the role of HCT in this disease is controversial. Histologic transformation to aggressive lymphoma occurs in up to 40 percent of patients, frequently leading to death within a few years of transformation.

### DEFINITION AND HISTORY

Follicular lymphoma (FL) is an indolent lymphoid neoplasm that is derived from mutated germinal center B cells and exhibits a nodular or follicular histologic pattern. It is typically composed of a mixture of

**Acronyms and Abbreviations:** ADCC, antibody-dependent cellular cytotoxicity; BR, bendamustine and rituximab; CDC, complement-dependent cytotoxicity; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CR, complete response; CVP, cyclophosphamide, vincristine, prednisone; FCM, fludarabine, cyclophosphamide, mitoxantrone; FDG, fluoro-2-deoxyglucose; FL, follicular lymphoma; FND, fludarabine, mitoxantrone (Novantrone), dexamethasone; GELF, Groupe d'Etudes des Lymphomes Folliculaires; Gy, gray; HLA, histocompatibility locus antigen; IFN, interferon; IPI, International Prognostic Index; KLH, keyhole limpet hemocyanin; LDH, lactate dehydrogenase; NHL, non-Hodgkin lymphoma; ORR, overall response rate; OS, overall survival; PCR, polymerase chain reaction; PET, positron emission tomography; PFS, progression-free survival; PR, partial remission; ProMACE/MOPP, prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, mechlorethamine, vincristine, procarbazine, prednisone; R-CHOP, rituximab plus CHOP; R-CVP, rituximab plus CVP; RIT, radioimmunotherapy; WHO, World Health Organization.

small, cleaved follicle center cells (centrocytes) and large noncleaved follicle center cells (centroblasts). The disease has masqueraded under multiple monikers, including “nodular lymphoma” in the Rappaport classification, and “follicle center cell lymphoma” in the Working Formulation.<sup>1</sup> The current World Health Organization (WHO) classification proposes the terms *follicular lymphoma, grades 1, 2, and 3* to differentiate cases based on the number of centroblasts per high-power microscopic field (the “Lymph Node Morphology and Lymphocyte Immunophenotype” section in this chapter has further information).<sup>2</sup>

### EPIDEMIOLOGY

FL accounts for approximately 20 to 25 percent of adult non-Hodgkin lymphomas (NHLs) in the United States, with an annual incidence of approximately 14,000 new cases per year.<sup>3,4</sup> FL is most common in North America and Western Europe and much less frequent in Eastern Europe, Asia, and Africa and in Americans of African descent.<sup>2</sup> The median age at diagnosis is 59 years, and the male-to-female ratio is 1:1.7. The disease is rare in persons younger than age 20 years, and pediatric cases appear to represent a separate disease entity that is typically localized, lacks the t(14;18) translocation and BCL2 expression, and has a very good prognosis.<sup>5,6</sup>

### CLINICAL FEATURES

#### SYMPTOMS AND SIGNS

Patients with FL usually present with painless diffuse lymphadenopathy. Less frequently, patients may have vague abdominal complaints, including pain, early satiety, and increasing girth, which may be caused by a large abdominal mass or hepatosplenomegaly. Approximately 10 percent of patients present with B symptoms (fever, drenching night sweats, or loss of 10 percent of body weight). The disease usually is widespread at presentation, with involvement of multiple lymph node-bearing sites, liver, and spleen. The marrow is involved in 40 to 70 percent of patients at diagnosis. FL may occasionally present with primary involvement of extranodal sites, such as the skin, gastrointestinal tract, ocular adnexa, and breast, but CNS disease is rare, unless histologic transformation to diffuse large B-cell lymphoma has occurred.<sup>2</sup>

### LABORATORY FEATURES

#### LYMPH NODE MORPHOLOGY AND LYMPHOCYTE IMMUNOPHENOTYPE

FL exhibits a predominantly nodular lymph node pattern; however, the neoplastic follicles are distorted, and as the disease progresses, the malignant follicles efface the nodal architecture (see Chap. 7, Fig. 7–18), commonly resulting in the development of areas of diffuse involvement, which may predominate histologically. The WHO has developed a three-grade system for classifying FL according to the proportion of centroblasts detected microscopically: Grade 1 lymphomas have 0 to 5 centroblasts, grade 2 lymphomas have 6 to 15 centroblasts, and grade 3 lymphomas have more than 15 centroblasts per high-power microscopic field (Fig. 10–1).<sup>2</sup> Grade 3 FL is further subdivided into grade 3A, in which some small centrocytes are present despite the predominance of centroblasts, and grade 3B, in which solid sheets of centroblasts are exclusively present and centrocytes are entirely absent.<sup>2</sup> Some, but not all, studies suggested that grades 1 and 2 lymphomas follow a more indolent course than grade 3 FL, and many authorities suggest that these lower grades should be treated more conservatively than grade 3 FL.<sup>7</sup> Other studies indicated a similar natural history for grades 1, 2, and 3A.<sup>8</sup>

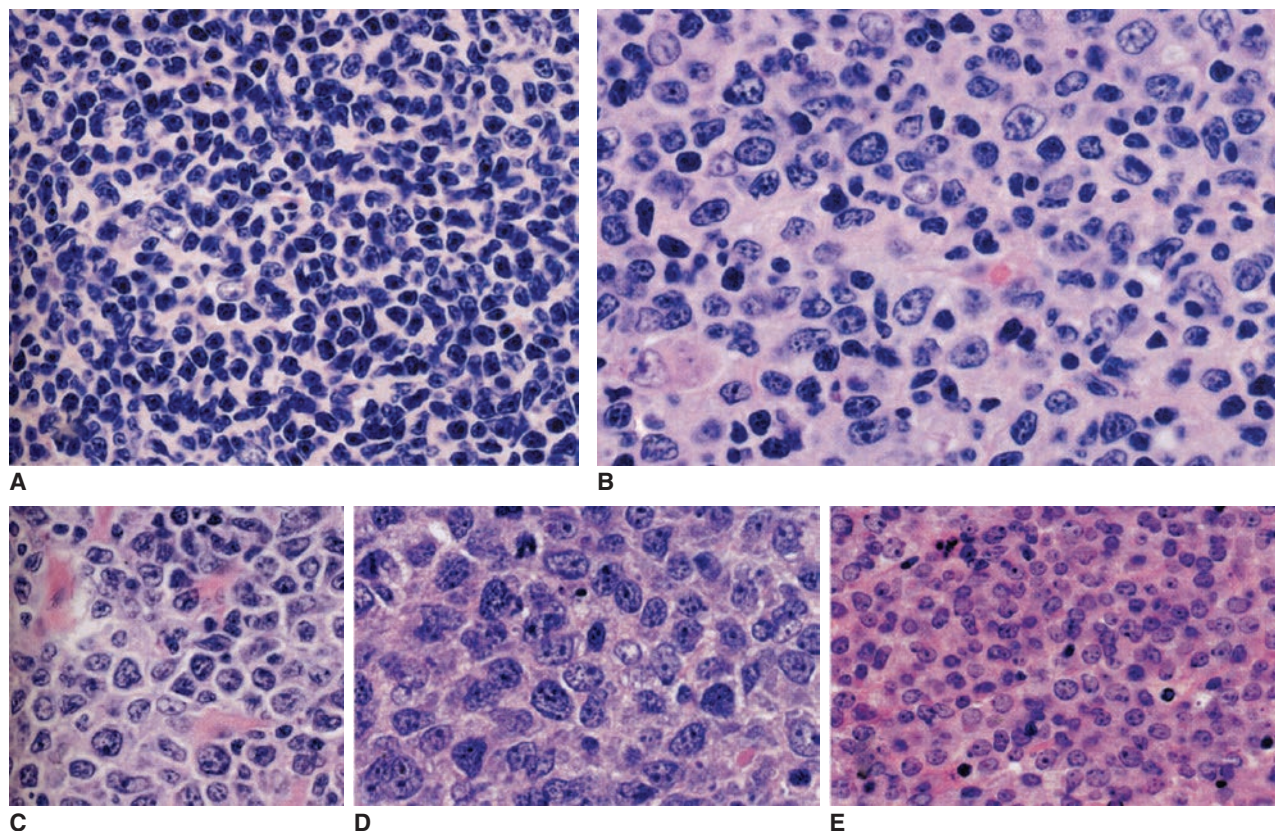


**Figure 10-1.** Follicular lymphoma grading is based on the relative proportions of small cells (centrocytes) and centroblasts (centroblasts). **A.** Grade 1 (0–5 centroblasts/high-powered field). **B.** Grade 2 (6–15 centroblasts/high-powered field). **C.** Grade 3A (>15 centroblasts/high-powered field). **D** and **E.** Grade 3B. See text for further definitions of grades 1, 2, 3A, and 3B. (Reproduced with permission from Harris NL, Swerdlow SH, Jaffe ES, et al: *Follicular lymphoma*, in *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, edited by Swerdlow SH, Campo E, Harris NL, et al: p 220–226. International Agency for Research on Cancer, Lyon, 2008.)

Nearly all authorities now agree, however, that grade 3B FL behaves aggressively and should be treated with anthracycline-containing regimens (e.g., rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone [R-CHOP]) similar to diffuse large B-cell lymphoma.<sup>2</sup> FL cells of all grades typically express monoclonal surface immunoglobulin; are positive for *BCL-2*, *BCL6*, and CD10; and express the pan-B-cell surface antigens CD19, CD20, CD22, and CD79a, but do not express CD5, CD23, CD11c, or CD43.

## CYTOGENETICS

The classic cytogenetic finding detected in FL is the t(14;18)(q32;q21) translocation that juxtaposes the *BCL-2* gene on band q21 of chromosome 18 with the immunoglobulin heavy-chain gene on band 32 of chromosome 14 (Fig. 10-2).<sup>9</sup> The immunoglobulin enhancer element results in amplified expression of the translocated gene product and thus overexpression of *BCL-2* protein leading to inhibition of apoptosis of affected B cells. Quantitative real-time polymerase chain reaction (PCR) assays on blood and marrow can determine the number of t(14;18)-expressing cells and may be useful in predicting the outcome of therapy. The t(14;18) translocation is found in approximately 85 percent of patients in the United States, but the translocation is present in a significantly lower percentage of Asian patients affected by FL. Detection of the t(14;18) translocation in lymphoid cells is neither necessary nor sufficient for the diagnosis of FL. Small numbers of B cells harboring the t(14;18) translocation can be detected in the blood of 25 to 75 percent of healthy individuals, as well as in reactive lymph nodes and tonsils if

a very sensitive nested or reverse-transcription PCR assay is employed.<sup>2</sup> Additional cytogenetic abnormalities are found in the cells of 90 percent of patients with FL. The finding of multiple cytogenetic abnormalities is commonly associated with higher histologic grade and with the probability of transformation to aggressive lymphoma. A recent large, high-resolution, genome-wide copy number analysis demonstrated that common recurrent chromosomal abnormalities include gains of chromosomes 2, 5, 6p, 7, 8, 12, 17q, 18, 21, and X and losses on 6q and 17p.<sup>10</sup> Frequent small abnormalities are also commonly observed, including

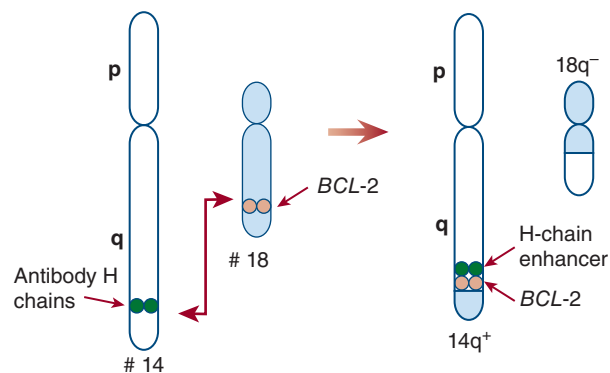


**Figure 10-2.** The t(14;18)(q32;q21) translocation juxtaposes the *BCL-2* gene on band q21 of chromosome 18 with the immunoglobulin heavy-chain gene on band 32 of chromosome 14.



losses of 1p36.33–p36.31, 6q23.3–q24.1, and 10q23.1–q25.1 and gains of 2p16.1–p15, 8q24.13–q24.3, and 12q12–q13.13. Copy number abnormalities more commonly observed in transformed FL include gains of 3q27.3–q28 and chromosome 11 and losses of 9p21.3 and 15q.<sup>10</sup> Important candidate genes whose expression is affected by these copy number abnormalities include TNFRSF14, PRDM16, TP73, and ARID1A on chromosome 1p36; BCL10 on chromosome 1p; REL and BCL11A on 2p16; BCL6 on chromosome 3q27; histocompatibility locus antigen (HLA)-B, HLA-C, CCND3, and PRDM1 on chromosome 6p21; TNFAIP3 or PERP on chromosome 6q23; CARD11 on chromosome 7p22; MYC on chromosome 8q24; CDKN2A or CDKN2B on chromosome 9p21; STAT6 on 12q13.3; and MDM2 on 12q15.<sup>10</sup>

## STAGING THE DISEASE

Evaluation of FL involves performance of a medical history, physical examination (with attention to the lymph nodes in Waldeyer ring and size and involvement of liver and spleen), laboratory testing (including a complete blood count, examination of the blood film and a differential white cell count, lactic dehydrogenase [LDH],  $\beta_2$ -microglobulin, comprehensive metabolic panel, serum uric acid level); lymph node biopsy; marrow aspiration and biopsy; flow cytometric analysis of blood, marrow, and lymph node cells; and computed tomography (CT) of the chest, abdomen, and pelvis.<sup>7</sup> Excisional lymph node biopsies are strongly preferred for the initial histologic diagnosis of FL, although in cases in which nodal masses are inaccessible, generous needle core biopsies may suffice. The diagnosis should not be established merely on the basis of flow cytometry of the blood or marrow or on cytologic examination of aspiration needle biopsies of lymph node or other tissue.<sup>11</sup> Hepatitis B serology should be assessed if rituximab therapy is contemplated, as hepatitis reactivation with rituximab may occasionally be life threatening. In selected circumstances, additional CT scans of the neck, measurement of the cardiac ejection fraction, serum protein electrophoresis, quantitative immunoglobulin measurement, and hepatitis C testing may be useful. Patients for whom chemotherapy is contemplated should receive counseling regarding contraception, fertility issues, and sperm or egg banking. The role of fluoro-2-deoxyglucose (FDG)–positron emission tomography (PET)/CT imaging in FL is rapidly evolving. Although PET/CT imaging was previously considered optional in FL, PET negativity at the completion of induction chemotherapy appears to be a powerful predictor of both progression-free survival (PFS) and overall survival (OS) in this disease.<sup>12</sup> It is also useful in confirming the absence of sites of disease that may harbor occult transformed lymphoma.<sup>12a</sup>

## PROGNOSTIC FACTORS

### CLINICAL AND LABORATORY VARIABLES

An international working group developed the International Prognostic Index (IPI) based on five independent variables (age, stage, LDH level, performance status, and number of extranodal sites) that affected OS of aggressive lymphoma patients treated with anthracycline-based combination chemotherapy.<sup>13</sup> The IPI was subsequently applied retrospectively to FL and found to be predictive of both OS and PFS for FL (as well as diffuse large B-cell lymphoma). Nevertheless, the IPI was considered to be suboptimal for segregating indolent lymphoma patients into prognostic categories because only 10 to 15 percent of patients with FL fall into the poor risk category using this index. To redress this deficiency, a French cooperative group conducted a detailed prognostic factor analysis of 4167 patients with FL diagnosed between 1985 and 1992 for whom prolonged followup was available to assess OS.<sup>14</sup> Five adverse prognostic factors were detected: age (>60 years vs. ≤60 years), Ann Arbor

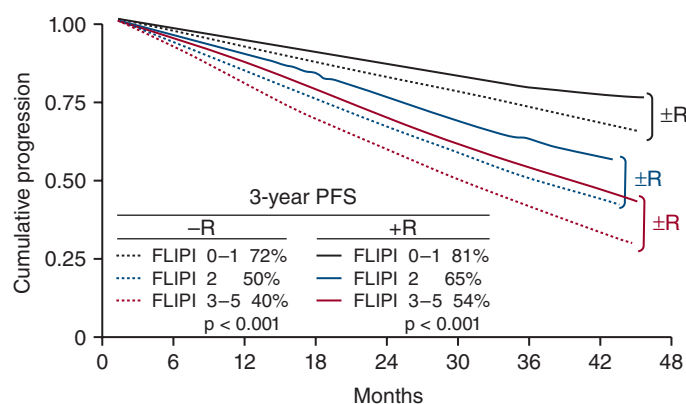


**Figure 10-3.** Progression-free survival (PFS) of 827 patients with follicular lymphoma (FL) stratified by the Follicular Lymphoma International Prognostic Index (FLIPI) into low risk (0 to 1 risk factors, 40 percent of patients, *black lines*), intermediate risk (2 risk factors, 33 percent of patients, *blue lines*), or high risk (3 to 5 risk factors, 27 percent of patients, *red lines*). Of the 827 patients, 267 were treated with chemotherapy regimens without rituximab (*dotted lines*), and 560 were treated with rituximab-containing regimens (*solid lines*). (Data from Federico M, Bellei M, Pro B: Revalidation of FLIPI in patients with follicular lymphoma registered in the F2 study and treated upfront with immunochemotherapy. *Proc Am Soc Clin Oncol* 25:443s, 2007.)

stage (III to IV vs. I to II), hemoglobin level (<120 g/L vs. ≥120 g/L), number of nodal areas (>4 vs. ≤4), and serum LDH level (high vs. normal). Three risk groups were defined: low risk (zero to one adverse factor, 36 percent of patients); intermediate risk (two adverse factors, 37 percent of patients, hazard ratio [HR] = 2.3); and poor risk (three or more adverse factors, 27 percent of patients, HR = 4.3). The Follicular Lymphoma International Prognostic Index (FLIPI) discriminated outcomes for FL better than the IPI, both in the original cohort and in later studies evaluating patients treated with modern combined rituximab-chemotherapy regimens (Fig. 10-3).<sup>15</sup> A revised version of the FLIPI index (FLIPI2) was subsequently proposed to address perceived deficiencies in the original model.<sup>16</sup> The FLIPI2 model is also based on assessment of five adverse risk factors: the presence or absence of an elevated  $\beta_2$ -microglobulin level, the longest diameter of the largest involved lymph node (>6 cm), presence of marrow involvement, hemoglobin level less than 12 g/dL, and age older than 60 years. Although several studies have demonstrated the superiority of the FLIPI2 model compared to the original FLIPI model, it has not been widely adopted in North America. A simpler prognostic model was developed based solely on the baseline serum LDH and  $\beta_2$ -microglobulin level and has been shown to be superior to the original FLIPI model and equivalent to the FLIPI2 model in prognostic power for predicting outcomes for FL patients.<sup>17,18</sup> Subsequently, a combination of clinical and molecular data have been advanced to comprise the m7-FLIPI, developed as a predictor of disease progression within 24 months (POD24).<sup>18a</sup> This approach is being assessed in prospective studies.

## GENOMICS OF FOLLICULAR LYMPHOMA

New molecular approaches are revolutionizing our understanding of the pathogenesis of FL and providing insights into the pathways that might be targeted in the future by rationally designed therapies. Early gene-expression profiling studies of biopsy specimens from patients with untreated FL identified two gene-expression signatures that allowed construction of a survival predictor enabling segregation of patients into four quartiles with disparate median lengths of survival (13.6, 11.1, 10.8, and 3.9 years), independent of clinical prognostic variables.<sup>19</sup> One signature

(“immune response 1”) was associated with a good prognosis and included genes encoding T-cell markers (e.g., *CD7*, *CD8B1*, *ITK*, *LEF1*, and *STAT4*) as well as genes that are highly expressed in macrophages (e.g., *ACTN1* and *TNFSF13B*). The “immune response 2” signature was associated with a poor prognosis and included genes known to be preferentially expressed in macrophages, dendritic cells, or both (e.g., *TLR5*, *FCGR1A*, *SEPT10*, *LGMN*, and *C3AR1*). Flow cytometry and cell sorting confirmed that these signatures reflected gene expression by nonmalignant tumor-infiltrating immune cells (CD19-negative cells) and not by the FL cells themselves (CD19-positive cells). The length of survival correlated with the molecular features of the nonmalignant immune cells present in the tumor at diagnosis and presumably reflected the robustness of the immune response mounted against the tumor.

“Next-generation sequencing” studies employing whole-genome or whole-exome sequencing, and targeted mutational analyses have demonstrated that somatic mutations in epigenetic regulators are present in almost all cases of FL, including *MLL2* (also known as *KMT2D*), a histone methyltransferase that is mutated in 89 percent of FL; *CREBBP* and *EP300*, two highly related histone and nonhistone acetyltransferases that act as transcriptional coactivators in multiple signaling pathways that are mutated in 30 percent and 11 percent of FL, respectively; *EZH2*, the catalytic subunit of *PRC2* that catalyzes trimethylation of lysine 27 on histone H3, a repressive chromatin mark that is mutated in 27 percent of FL; and *MEF2B*, a calcium-regulated gene that cooperates with *CREBBP* and *EP300* in acetylating histones that is mutated in 15 percent of FL.<sup>20–24</sup> Other mutational targets include genes involved in immune modulation ( $\beta_2$ -microglobulin, *CD58*, and *TNFRSF14*); Janus kinase (*JAK*)-signal transducer and activator of transcription (*STAT*) signaling (*SOCS1* and *STAT6*); and B-cell receptor–nuclear factor (*NF*)- $\kappa$ B signaling (*BCL10*, *CARD11*, and *CD79B*).<sup>22</sup> Mutations in *CREBBP* and *EP300* genes are usually heterozygous, suggesting a haploinsufficient role in tumor suppression, apparently by impairing acetylation-mediated inactivation of the *BCL6* oncoprotein and activation of the *p53* tumor suppressor.<sup>21</sup> A unifying model of the genetic evolution of FL postulates that the t(14;18) translocation serves as a “founder mutation” that is succeeded by development of additional “driver mutations” (e.g., *CREBBP*), irrelevant “passenger” mutations, and accelerator mutations (*TNFRSF14*).<sup>22,23</sup> Disagreement exists over whether *MLL2/KMT2D* mutations are driver or accelerator mutations.<sup>22,23</sup> Mutations in *EBF1* and in regulators of *NF*- $\kappa$ B signaling (e.g., *MYD88*, *TNFAIP3*) appear to be acquired at the time of histologic transformation to diffuse large B-cell lymphoma.<sup>22</sup>

## ● THERAPY

### LIMITED STAGE I OR II FOLLICULAR LYMPHOMA

#### Radiotherapy

Patients with stage I or II FL represent only 10 to 30 percent of cases in most series.<sup>2,4</sup> Standard management for stage I or limited contiguous stage II FL involves the administration of involved-field radiotherapy (35 to 40 Gray [Gy]).<sup>7</sup> Adjuvant chemotherapy does not appear to improve survival in this setting, although some studies suggested that combined chemoradiotherapy may improve PFS. A retrospective review of 177 patients with stage I or II and grade 1 or 2 FL reported a median survival of 14 years following radiation therapy as a single modality.<sup>25</sup> Approximately 50 percent of the patients were relapse free after 5 to 10 years.

#### Observation

Excellent survival has also been observed in highly selected patients with early stage FL who received no initial therapy.<sup>26</sup> In a group of 43 patients, 56 percent were free from the requirement for treatment for at least

10 years, and 86 percent were alive 10 years after diagnosis. Based on this study, many authorities have concluded that “watchful waiting” is an acceptable alternative to radiotherapy for stage I or II FL. A watchful waiting approach may be particularly appropriate for certain variants of FL, such as FL presenting in the small intestine, which pursues a remarkably indolent course, rarely exhibits progressive growth, very rarely disseminates (two of 63 patients), and does not transform to high-grade disease.<sup>27</sup>

#### Chemoimmunotherapy

A large observational study, the National LymphoCare Study, assessed the outcomes of 471 patients with stage I FL according to treatment administered, including rituximab plus chemotherapy (28 percent of patients); radiotherapy alone (27 percent); observation (17 percent); systemic therapy plus radiotherapy (13 percent); rituximab monotherapy (12 percent); and other treatments (3 percent).<sup>28</sup> This large, prospectively enrolled group of patients showed that national guidelines endorsing radiotherapy alone for stage I FL were not followed by practicing clinicians in the majority of cases. All treatment approaches resulted in excellent outcomes, though PFS was significantly better after a median followup of 57 months in patients treated with either rituximab plus chemotherapy (84 percent) or systemic therapy plus radiotherapy (96 percent) than in patients receiving radiotherapy alone (68 percent).<sup>28</sup> This study challenges the paradigm that radiotherapy alone should be the standard of care for patients with early stage indolent lymphoma, although the observational nature of this study, without randomization to treatment arm and the absence of differences in OS, attenuates the impact of the study.

### ADVANCED-STAGE FOLLICULAR LYMPHOMA

#### Observation Alone

Many patients with FL, particularly grades 1 or 2, will exhibit an indolent, asymptomatic course despite the absence of therapy. Because there is no conclusive evidence that survival of FL patients is improved by immediate institution of therapy or that conventional management (other than allogeneic stem cell transplantation) can cure the disease, a “watch-and-wait” approach is often recommended for patients with extensive stage II or stage III or IV FL. In one study, survival was 82 percent at 5 years and 73 percent at 10 years after an initial strategy of observation alone, and the median time until therapy was required was 3 years.<sup>29</sup> Spontaneous regressions occurred in 23 percent of untreated patients. No differences in survival were observed in a trial of 309 patients randomized to initial watchful waiting or to chlorambucil.<sup>30</sup> In another trial, patients were randomized to either watchful waiting or to immediate aggressive combination chemotherapy with prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide, mechlorethamine, vincristine, procarbazine, prednisone (ProMACE/MOPP) chemotherapy followed by total nodal irradiation.<sup>31</sup> The OS rates for the two groups were similar, although the disease-free survival rate was naturally higher in the patients treated with combined modality therapy. Criteria established by the Groupe d'Etudes des Lymphomes Folliculaires (GELF) are useful to identify patients who may benefit from intervention rather than watchful waiting. These criteria suggest that treatment is likely to be required for patients with a maximum diameter of any site of disease greater than 7 cm, more than three nodal sites greater than 3 cm in diameter, systemic B symptoms, a spleen size greater than 16 cm, pleural effusions, local compressive symptoms, circulating lymphoma cells, or cytopenias as a result of the lymphoma.<sup>32</sup>

#### Single-Agent Chemotherapy

FL patients can be palliated effectively with a variety of single chemotherapy agents (Table 10–1). Responses to single-agent therapy, such as

**TABLE 10-1.** Therapeutic Regimens for Follicular Lymphoma

| Agent(s)                   | Dose                                 | Route           | Days(s) of Treatment | Repeat Cycle at Day |
|----------------------------|--------------------------------------|-----------------|----------------------|---------------------|
| <b>SINGLE AGENTS</b>       |                                      |                 |                      |                     |
| Chlorambucil               | 0.08–0.12 mg/kg                      | PO              | Daily                |                     |
|                            | or 0.4–1.0 mg/kg                     | PO              | 1                    | 28                  |
| Cyclophosphamide           | 50–100 mg/m <sup>2</sup>             | PO              | Daily                |                     |
|                            | or 300 mg/m <sup>2</sup>             | PO              | 1–5                  | 28                  |
| Fludarabine                | 25 mg/m <sup>2</sup> /day            | IV              | 1–5                  | 28                  |
| Cladribine                 | 0.1 mg/kg/day                        | IV (continuous) | 1–7                  | 28                  |
|                            | or 0.14 mg/kg/day                    | IV (2 h)        | 1–5                  | 28                  |
| Bendamustine               | 70–120 mg/m <sup>2</sup> /day        | IV              | 1, 2                 | 21 or 28            |
| Rituximab                  | 375 mg/m <sup>2</sup> /day           | IV              | 1, 8, 15, 22         |                     |
| <b>COMBINATION THERAPY</b> |                                      |                 |                      |                     |
| Stanford CVP               |                                      |                 |                      |                     |
| Cyclophosphamide           | 400 mg/m <sup>2</sup>                | PO              | 1–5                  | 21                  |
| Vincristine                | 1.4 mg/m <sup>2</sup> (maximum 2 mg) | IV              | 1                    | 21                  |
| Prednisone                 | 100 mg/m <sup>2</sup>                | PO              | 1–5                  | 21                  |
| R-CVP                      |                                      |                 |                      |                     |
| Rituximab                  | 375 mg/m <sup>2</sup>                | IV              | 1                    | 21                  |
| Cyclophosphamide           | 750–1000 mg/m <sup>2</sup>           | IV              | 1                    | 21                  |
| Vincristine                | 1.4 mg/m <sup>2</sup> (maximum 2 mg) | IV              | 1                    | 21                  |
| Prednisone                 | 100 mg                               | PO              | 1–5                  | 21                  |
| R-CHOP                     |                                      |                 |                      |                     |
| Rituximab                  | 375 mg/m <sup>2</sup>                | IV              | 1                    | 21                  |
| Cyclophosphamide           | 750 mg/m <sup>2</sup>                | IV              | 1                    | 21                  |
| Doxorubicin                | 50 mg/m <sup>2</sup>                 | IV              | 1                    |                     |
| Vincristine                | 1.4 mg/m <sup>2</sup>                | IV              | 1                    |                     |
| Prednisone                 | 100 mg                               | PO              | 1–5                  |                     |
| FND                        |                                      |                 |                      |                     |
| Fludarabine                | 25 mg/m <sup>2</sup>                 | IV              | 1–3                  | 28                  |
| Mitoxantrone               | 10 mg/m <sup>2</sup>                 | IV              | 1                    |                     |
| Dexamethasone              | 20 mg                                | IV or PO        | 1–5                  |                     |

FND, fludarabine, mitoxantrone (Novantrone), dexamethasone.

with chlorambucil, a nucleoside analogue, or bendamustine, range from 70 to 90 percent and may last for several years.<sup>30,33</sup>

### Monoclonal Antibody Therapy

Rituximab is a human-mouse chimeric monoclonal antibody that binds to the CD20 antigen that is expressed on nearly all normal and malignant B cells but not on other human tissues. After binding to B cells, rituximab induces cell death via antibody-dependent cellular cytotoxicity (ADCC), complement fixation (complement-dependent cytotoxicity [CDC]), and induction of apoptosis and by facilitating cross-presentation of lymphoma-associated antigens by dendritic cells. Rituximab was approved by the FDA for therapy of indolent lymphomas based on the results of a pivotal trial that evaluated treatment of 166 patients with relapsed or refractory indolent lymphoma with four weekly infusions of 375 mg/m<sup>2</sup>. The response rate was 48 percent, including a 6 percent complete response (CR) rate and

a median time to progression of approximately 1 year.<sup>34</sup> The response rate to first-line therapy with rituximab in newly diagnosed FL is approximately 70 to 75 percent, with a complete remission rate of 18 to 27 percent.<sup>35,36</sup>

A second response to rituximab may be achieved in 40 percent of patients who relapse after an initial remission to rituximab.<sup>37</sup> Extended courses of rituximab or “rituximab maintenance” therapy have become popular. Various schedules are employed, including administration of one dose of 375 mg/m<sup>2</sup> every 2 months for 2 years (usually as part of frontline therapy), one dose every 3 months for 2 years (for relapsed patients), four doses every 6 months for 2 years, or one dose every 2 months for four doses.<sup>38–42</sup> No comparative studies of these disparate “maintenance” rituximab regimens have been performed. A subcutaneously administered formulation of rituximab (with human hyaluronidase) is available for use for administrations subsequent to the initial intravenous infusion. Efficacy and safety are comparable to the intravenous formulation, with the exception



**TABLE 10–2.** Selected Randomized Studies of Chemotherapy Alone Versus Rituximab Plus Chemotherapy for First-Line Therapy of Follicular Lymphoma

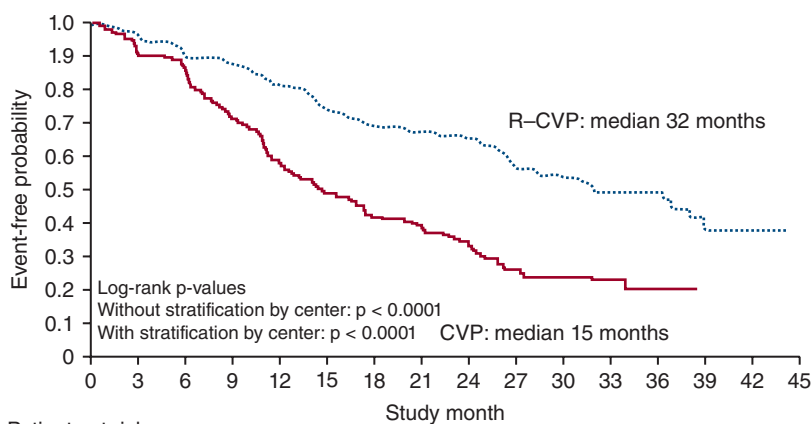
| Study                   | Treatment, No. of Patients       | Median Followup (Months) | ORR (%) | CR (%) | Median TTP/TTF/EFS (Months) | OS (%)               |
|-------------------------|----------------------------------|--------------------------|---------|--------|-----------------------------|----------------------|
| Marcus <sup>45</sup>    | CVP, 159                         | 53                       | 57      | 10     | 15                          | 77                   |
|                         | R-CVP, 162                       |                          | 81      | 41     | 34                          | 83                   |
|                         |                                  |                          |         |        | $p < 0.0001$                | $p = 0.0290$         |
| Hiddemann <sup>46</sup> | CHOP, 205                        | 18                       | 90      | 17     | 29                          | 74                   |
|                         | R-CHOP, 223                      |                          | 96      | 20     | NR                          | 87                   |
|                         |                                  |                          |         |        | $p < 0.001$                 | $p = 0.016$          |
| Herold <sup>47</sup>    | MCP, 96                          | 47                       | 75      | 25     | 26                          | 74                   |
|                         | R-MCP, 105                       |                          | 92      | 50     | NR                          | 87                   |
|                         |                                  |                          |         |        | $p < 0.0001$                | $p = 0.0096$         |
| Bachy <sup>43</sup>     | CHVP-IFN, 183                    | 100                      | 73      | 63     | 34                          | 69                   |
|                         | R-CHVP-IFN, 175                  |                          | 84      | 79     | 66                          | 78 at 8 years        |
|                         |                                  |                          |         |        | $p = 0.0004$                | $p = 0.076$          |
| Hochster <sup>44</sup>  | CVP, 158                         | 36                       | 82      | 22     | 16                          | 92                   |
|                         | CVP + rituximab maintenance, 153 |                          | 86      | 37     | 59                          | 86 at 3 years        |
|                         |                                  |                          |         |        | $p = 4.4 \times 10^{-10}$   | $p = 0.05$ one sided |

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CHVP, cyclophosphamide, doxorubicin, teniposide, prednisone; CR, complete response; CVP, cyclophosphamide, vincristine, prednisone; EFS, event-free survival; IFN, interferon; MCP, mitoxantrone, chlorambucil, prednisone; ORR, overall response rate; OS, overall survival; R, rituximab; TTF, time to treatment failure; TTP, time to progression.

of injection site reactions and faster treatment times being associated with subcutaneous injection.<sup>42a</sup> This route of administration is essentially interchangeable with intravenous treatment in most settings and may allow for greater patient and nursing convenience. Several newer, humanized or fully human anti-CD20 monoclonal antibodies (ofatumumab, veltuzumab, obinutuzumab) have been engineered to exhibit superior ADCC, CDC, or improved induction of cell death. All are undergoing clinical trials to determine if they are superior to rituximab, and one of them, obinutuzumab, is approved for therapy of chronic lymphocytic leukemia (CLL) and FL in combination with chemotherapy. “Biosimilar” CD20 antibodies are also emerging as an alternative to rituximab.

### Rituximab Plus Chemotherapy

The introduction of rituximab into treatment protocols for FL has revolutionized the management of this disease. Multiple randomized, controlled clinical trials have documented the superiority of combining rituximab with chemotherapy compared to the use of chemotherapy alone in terms of overall response rates (ORRs), CR rates, event-free survival (EFS), PFS, and OS (Table 10–2).<sup>43–47</sup> In one study, induction therapy consisting of eight cycles of rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP) was compared to eight cycles of CVP without rituximab in 321 patients with newly diagnosed FL (Fig. 10–4).<sup>48</sup> R-CVP was superior to CVP alone in terms of ORR (81 percent vs. 57 percent),



Patients at risk:

|       |     |     |     |     |     |     |     |     |    |    |    |    |    |    |   |   |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|----|----|---|---|
| CVP   | 159 | 140 | 129 | 109 | 87  | 75  | 64  | 58  | 46 | 28 | 21 | 12 | 5  | 0  | 0 | 0 |
| R-CVP | 162 | 156 | 144 | 140 | 131 | 119 | 111 | 106 | 95 | 68 | 50 | 32 | 20 | 10 | 2 | 0 |

**Figure 10–4.** Time to disease progression, relapse, or death after a median followup of 30 months among 321 patients with grade 1 or 2 follicular lymphoma assigned to chemotherapy with CVP (cyclophosphamide, vincristine, prednisone) or with R-CVP (rituximab plus cyclophosphamide, vincristine, prednisone). Solid line represents CVP; dotted line, R-CVP. (Reproduced with permission from Marcus R, Imrie K, Belch A, et al: CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. *Blood* 2005 Feb 15;105(4):1417-1423.)

CR rate (41 vs. 10 percent), time to progression (34 vs. 15 months), time to treatment failure (27 vs. 7 months), and OS (83 vs. 77 percent at 4 years,  $p = 0.029$ ).<sup>45</sup> Similarly, R-CHOP was compared to CHOP for first-line treatment of 428 patients with advanced-stage FL. R-CHOP exhibited a superior ORR (96 vs. 90 percent), time to treatment failure ( $p < 0.001$ ), duration of response ( $p = 0.001$ ), and OS ( $p = 0.016$ ) compared to CHOP alone.<sup>46</sup> Similar benefits have also been reported for the addition of rituximab to other regimens for both frontline therapy and relapsed FL.<sup>41,47,49,50</sup> The selection of the “optimal” chemotherapy regimen to combine with rituximab remains hotly contentious.<sup>51</sup> Two large international phase III randomized studies compared the efficacy and toxicity of R-CVP, R-CHOP, and rituximab-fludarabine-based therapy, which until recently were the three most commonly employed regimens for frontline therapy of FL. Both trials demonstrated superior PFS for patients treated with R-CHOP or rituximab-fludarabine regimens than with R-CVP, although OS did not differ. Both studies also concluded that regimens with fludarabine and rituximab had significantly more hematologic toxicity and a higher risk of secondary malignancies than either R-CVP or R-CHOP, making fludarabine-based regimens less desirable. The investigators conducting these trials concluded that R-CHOP was the preferred regimen for FL, although some oncologists are hesitant to routinely employ R-CHOP in patients with indolent lymphomas because of its greater toxicity, including a 1 to 2 percent risk of cardiomyopathy, and the absence of a demonstrated OS advantage. These risks may be particularly justifiable, however, for patients with grade 3 FL, as many authorities believe anthracycline-based regimens may be curable for high-grade FL, which the National Comprehensive Cancer Network (NCCN) guidelines advise be treated identically to diffuse large B-cell lymphoma.<sup>7</sup> To further amplify the controversy, two recent randomized trials comparing bendamustine plus rituximab (BR) with R-CHOP showed similar efficacy for the two regimens and less toxicity for BR.<sup>52,53</sup> Although these studies have been criticized for methodologic flaws, they have been highly influential and have catapulted BR to prominence as the most popular frontline induction regimen for FL, with as many as 65 to 70 percent of FL patients in the United States and Europe currently receiving this regimen for frontline therapy.

### Maintenance Rituximab

Despite the ability to induce long-lasting remissions in the majority of FL patients treated with chemoimmunotherapy regimens as outlined previously, most patients will eventually relapse. Several strategies have emerged to prolong the remission durations and to delay lymphoma recurrence. The most popular approach is to administer extended courses of rituximab, also known as rituximab “maintenance,” to forestall reappearance of FL. The PRIMA trial randomized 1217 patients with FL to either maintenance rituximab (375 mg/m<sup>2</sup> every 2 months for 2 years) or no maintenance therapy, following frontline induction therapy with R-CVP, R-CHOP, or rituximab, fludarabine, cyclophosphamide, and mitoxantrone (R-FCM).<sup>42</sup> This study convincingly demonstrated the superiority of 2 years of maintenance rituximab (PFS 74.9 percent) compared to observation alone after induction (PFS, 57.6 percent,  $p < 0.0001$ ), regardless of which induction chemotherapy regimen was used. Despite the marked improvement in PFS afforded by maintenance rituximab in PRIMA, however, there was no significant difference in OS between the rituximab maintenance and observation arms. Similar improvements in PFS but not OS have also been demonstrated for maintenance rituximab given for 2 years following rituximab monotherapy in the frontline setting<sup>30</sup> and following CHOP or R-CHOP induction in the relapsed setting.<sup>54</sup>

### Obinutuzumab with Chemotherapy

Type II anti-CD20 antibodies are engineered for enhanced direct killing and antibody-dependent cellular cytotoxicity (with less complement activation)

in comparison with rituximab. One such glyco-engineered anti-CD20 antibody, obinutuzumab, was assessed in combination with bendamustine—versus bendamustine alone—in patients with rituximab-refractory indolent lymphoma in the GADOLIN study.<sup>54a</sup> While the obinutuzumab-containing arm also contained maintenance dosing, and the bendamustine alone arm did not include rituximab, improvements in PFS were demonstrated through the addition of obinutuzumab to bendamustine in this setting. Adverse events with obinutuzumab include infusion reactions and cytopenias.

The GALLIUM study compared obinutuzumab with chemotherapy plus obinutuzumab maintenance for 2 years versus rituximab with chemotherapy plus rituximab maintenance for 2 years in previously untreated FL patients.<sup>54b</sup> PFS was improved in the obinutuzumab-containing arm at 3 years (80.0 vs. 73.3 percent), though no differences in OS were noted. An increased rate of death was noted in the bendamustine-containing arms (regardless of antibody) versus the CHOP-containing arms, provoking speculation on the potential risks of maintenance anti-CD20 antibody therapy with bendamustine treatment. Further analysis of these data and related issues is ongoing.

### Radioimmunotherapy

Radiolabeled monoclonal antibodies targeting lymphoma-associated cell surface antigens, including idiotypic immunoglobulin, CD20, CD22, and HLA-DR, have emerged as effective and safe therapeutic agents for patients with FL.<sup>55,56</sup> Two radioimmunoconjugates targeting the CD20 antigen, <sup>131</sup>iodine-tositumomab (Bexxar) and <sup>90</sup>yttrium-ibritumomab tiuxetan (Zevalin), have been extensively tested in indolent lymphomas.<sup>57–59</sup> Radioimmunotherapy (RIT) is a potentially attractive therapeutic option for lymphomas because (1) many high-quality antibodies are available targeting pan-B-cell antigens expressed at high levels on lymphoma cells, (2) lymphomas are exquisitely sensitive to radiotherapy, and (3) cross-fire radiotherapy from  $\beta$  particles emitted by decaying radionuclides on targeted lymphoma cells can kill neighboring antigen-negative tumor cells (or inaccessible cells deep in tumor clumps), which would escape killing by nonradioactive antibodies. Several trials have demonstrated ORRs of 50 to 80 percent and CR rates of 15 to 40 percent in patients with relapsed or refractory indolent lymphoma treated with either <sup>131</sup>iodine-tositumomab or <sup>90</sup>yttrium-ibritumomab tiuxetan.<sup>57,59,60</sup> In a randomized study comparing treatment of patients with relapsed FL with either <sup>90</sup>Y-ibritumomab tiuxetan or rituximab, the ORR (86 vs. 55 percent) and the CR rate (30 vs. 15 percent) were both statistically superior in the group treated with the radioimmunoconjugate.<sup>59</sup> Similarly, <sup>131</sup>I-tositumomab was compared with unlabeled tositumomab in a randomized trial of relapsed indolent lymphoma, and both the ORR (55 vs. 19 percent) and the CR rate (33 vs. 8 percent) were higher in patients receiving the radiolabeled antibody.<sup>61</sup>

Six phase II studies have studied frontline RIT for patients with newly diagnosed FL, with RIT either as a single agent or in combination with various chemotherapy regimens, including CVP, CHOP, and fludarabine. In all six studies, outstanding ORR rates (90 to 100 percent) and CR rates (50 to 96 percent) were observed with first-line RIT, with median PFSs in excess of 5 years in several of the studies.<sup>62–64</sup> A phase III randomized study evaluated the utility of consolidation therapy with <sup>90</sup>Y-ibritumomab tiuxetan for patients with FL in remission after frontline chemotherapy.<sup>65</sup> In this trial, 414 patients in either partial or complete remission after a variety of chemotherapy induction regimens (chlorambucil, CVP, CHOP, fludarabine, or rituximab combinations) were randomized to either consolidation with RIT or to no consolidation. RIT dramatically improved the median PFS in the total patient population (36.5 vs. 13.3 months,  $p < 0.0001$ ), and this advantage was observed regardless of whether patients were in partial remission (PR; 29.3 vs. 6.2 months,  $p < 0.0001$ ) or CR (53.9 vs. 29.5 months,  $p = 0.015$ ) at the time of consolidation. Furthermore, RIT consolidation converted

77 percent of patients who were in PR after induction chemotherapy to CR following RIT. However, a recent study comparing six cycles of R-CHOP with six cycles of CHOP (without rituximab) followed by a single dose of  $^{131}\text{I}$ -tositumomab (CHOP-RIT) demonstrated similar outcomes in both treatment arms.<sup>17</sup>

The major toxicity of RIT is myelosuppression, with cytopenic nadirs occurring 4 to 7 weeks after treatment and requiring 2 to 4 weeks for recovery. Growth factor administration and transfusions are required in approximately 20 percent of patients. Human antimouse antibodies (HAMAs) may develop in the serum of approximately 1 percent of patients treated with  $^{90}\text{Y}$ -labeled ibritumomab and in 10 percent of patients treated with  $^{131}\text{I}$ -labeled tositumomab. A potential long-term concern with both radiolabeled antibody formulations is the potential development of myelodysplasia and acute leukemia as late complications. Hypothyroidism may also occur as a delayed toxicity of  $^{131}\text{I}$ -labeled tositumomab in approximately 10 percent of patients.

### Phosphoinositide 3-Kinase Inhibitors

The phosphoinositide 3-kinase (PI3K)/Protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway is relevant to cell signaling and proliferation in a variety of malignancies, including B-cell lymphomas and leukemias. The PI3K delta inhibitor idelalisib has been evaluated in various tumors, leading to its approval in indolent B-cell malignancies and CLL. In a phase II trial, 125 patients with indolent NHL were treated orally with idelalisib 150 mg twice daily until disease progression.<sup>65a</sup> Subjects had rituximab-refractory and alkylator-refractory disease and had received a median of four prior therapies. The ORR was 57 percent (6 percent CR), and median PFS was 11 months. Principal toxicities included neutropenia, liver enzyme abnormalities, pneumonia, and gastrointestinal toxicity, including diarrhea and colitis. While most toxicities were reversible upon dose modifications, subsequent studies showed a significant risk of infectious complications (including pneumocystis and cytomegalovirus) when idelalisib was combined in regimens with other agents. Idelalisib has activity in patients with high-risk FL, with high risk defined by an early relapse (within 2 years) following initial chemimmunotherapy.<sup>65b</sup> Other PI3K inhibitors, including copanlisib and umbralisib, have also been shown to be active in indolent and other B-cell lymphomas and are undergoing further study.

### Interferon- $\alpha_2$

Interferon (IFN)  $\alpha_2$  has been studied in 10 large phase III studies evaluating its utility in both the induction phase of treatment and maintenance therapy. A meta-analysis of 1922 newly diagnosed patients with FL treated in these trials concluded that the addition of IFN- $\alpha_2$  to induction chemotherapy did not significantly influence response rates but did show a significant difference in favor of IFN- $\alpha_2$  with regard to survival.<sup>66</sup> Results differed greatly from trial to trial, and further analyses were carried out to define the circumstances in which IFN- $\alpha_2$  prolonged OS. The survival advantage was seen when IFN- $\alpha_2$  was given (1) in conjunction with relatively intensive initial chemotherapy ( $p = 0.00005$ ), (2) at a dose of 5 million units or greater ( $p = 0.000002$ ), (3) at a cumulative dose of 36 million units or greater per month ( $p = 0.000008$ ), and (4) when given with induction chemotherapy rather than as maintenance therapy ( $p = 0.004$ ).<sup>66</sup> With regard to remission duration, there was also a significant difference in favor of IFN- $\alpha_2$ , irrespective of the intensity of chemotherapy used, IFN dose, or whether IFN was given as a maintenance strategy or with chemotherapy. Despite these salutary findings, IFN is rarely employed to treat FL in the United States because of its unfavorable toxicity profile (asthenia, fatigue, flu-like symptoms, cytopenias) and because of the perception that rituximab confers similar or superior advantages with much less toxicity.

### Idiotype Vaccines

The idiotypic immunoglobulin protein expressed on the surface of B lymphoma cells represents a true tumor-specific antigen and is an ideal target for immunotherapeutic strategies. Several groups have reported favorable phase II trials using idiotypic vaccines produced either by rescue hybridoma fusions or recombinant DNA approaches. The idiotypic immunoglobulin in most of the vaccines is coupled to keyhole limpet hemocyanin (KLH) and administered with sargramostim (granulocyte-macrophage colony-stimulating factor) to enhance immunogenicity. Specific immune responses are generated in approximately 50 percent of immunized FL patients who are in complete remission at the time of vaccination. Patients exhibiting immune responses to the vaccine experience longer remission durations and superior survival compared to patients failing to mount immune responses to the vaccine. Three phase III randomized trials have been conducted using idiotypic vaccination following induction therapy. Two of these studies found no statistical differences in PFS between an idiotypic KLH vaccine and a control vaccine.<sup>67,68</sup> The third study reported an improved disease-free survival for specifically vaccinated patients,<sup>69</sup> although the study has been criticized for a variety of methodologic imperfections.<sup>68</sup>

### Hematopoietic Stem Cell Transplantation

The role of high-dose chemoradiotherapy and hematopoietic stem cell transplantation in the management of patients with FL remains highly controversial. Proponents of autologous stem cell transplantation for indolent NHL note the favorable outcome of a collaborative study of 121 adult patients conducted by St. Bartholomew's Hospital and the Dana-Farber Cancer Institute, where an apparent plateau in the remission duration curve was observed in 48 percent of patients with a median followup of 13.5 years.<sup>70</sup> Survival was longer in patients transplanted in second remission compared to those transplanted later in their disease course. The value of autologous stem cell transplantation was also tested in a randomized trial of 89 patients with relapsed FL (Fig. 10-5). Transplanted patients experienced a marked advantage in PFS and a

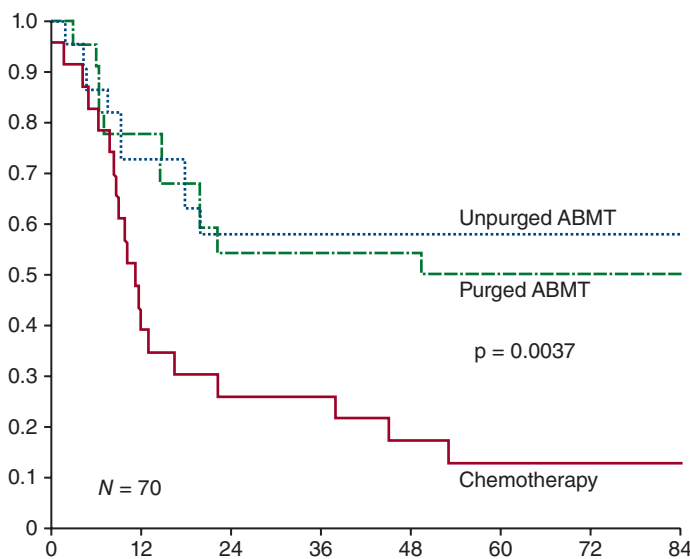


**Figure 10-5.** Progression-free survival of 70 patients with relapsed follicular lymphoma randomized to either conventional chemotherapy (solid line) or high-dose chemoradiotherapy with autologous marrow transplantation using either marrow purged to remove tumor cells (long dashes) or unpurged marrow (short dashes). ABMT, autologous bone marrow transplantation. (Reproduced with permission from Schouten HC, Qian W, Kvaloy S, et al: High-dose therapy improves progression-free survival and survival in relapsed follicular non-Hodgkin's lymphoma: Results from the randomized European CUP trial. *J Clin Oncol* 2003 Nov 1;21(21):3918-3927.



marginal OS advantage compared to patients randomized to continued conventional salvage chemotherapy without transplantation.<sup>71</sup> When used as part of initial therapy for high-risk patients, randomized studies demonstrated a prolongation of PFS, but no improvement in OS.<sup>72,73</sup> Adverse outcomes associated with autologous stem cell transplantation include treatment-related mortality (3 to 5 percent) and a substantial increase in the incidence of secondary myelodysplasia and acute myelogenous leukemia, occurring in 7 to 19 percent of patients, particularly if total-body irradiation is employed in the conditioning regimen.

Allogeneic transplantation affords long-term PFS for approximately 40 to 50 percent of patients with relapsed FL, but widespread adoption of this approach has been hampered by transplant-related mortality, ranging from 20 to 40 percent. Consequently, careful patient selection and informed consent are essential. When allogeneic and autologous stem cell transplantation are compared for patients with relapsed FL, the long-term survival rates appear to be comparable.<sup>74,75</sup> Autologous stem cell transplantation is associated with a greater likelihood of dying from recurrent disease, and allogeneic stem cell transplantation results in a higher frequency of death from graft-versus-host disease, infection, and venoocclusive disease. Nonmyeloablative and reduced-intensity allogeneic transplantation conditioning regimens have been developed to exploit the benefit of a graft-versus-lymphoma effect while minimizing transplant-related morbidity and mortality. Preliminary results of this approach are encouraging, with 52 to 85 percent OS and 43 to 83 percent PFS after 3 to 11 years, with 8 to 43 percent nonrelapse mortality.<sup>76,77</sup>

## TRANSFORMED FOLLICULAR LYMPHOMA

As many as 30 to 40 percent of patients with FL undergo documented transformation to a more aggressive histology, usually diffuse large B-cell lymphoma, with an annual rate of transformation of approximately 3 percent (Fig. 10–6A). Clinically, histologic transformation is characterized by the sudden explosive growth of a single lymph node site (or extranodal mass). FDG-PET scanning can identify areas of high uptake that may suggest transformation, prompting a targeted biopsy. Anthracycline-based chemotherapy (e.g., R-CHOP) is the most appropriate therapy for patients experiencing transformation; however, most studies indicated that

the prognosis is poor despite aggressive management. Whereas 50 to 65 percent of patients presenting with *de novo* diffuse large B-cell lymphoma are cured with R-CHOP chemotherapy, less than 10 percent of patients with diffuse large B-cell lymphoma arising by transformation from previously treated FL will be cured by this regimen (Fig. 10–6B). Most series report median survivals of 6 to 20 months for patients undergoing transformation,<sup>29,78–80</sup> although one recent study reported a 50-month median survival, with particularly good survival in patients experiencing transformation more than 18 months after initial diagnosis of FL.<sup>81</sup> Because of the historically poor outcome of chemotherapy alone, some authorities advise either autologous or allogeneic stem cell transplantation following induction of remission with R-CHOP. A recent multicenter cohort study of 172 patients with transformed FL concluded that patients undergoing autologous stem cell transplantation had better outcomes than those treated with rituximab-containing chemotherapy alone. However, allogeneic transplantation did not improve outcomes compared with rituximab-containing chemotherapy because of high rates of transplant-related mortality.<sup>82</sup>

## A PRAGMATIC APPROACH TO THERAPY OF FOLLICULAR LYMPHOMA

There is currently little consensus among lymphoma experts with regard to the optimal management of patients with either front-line or relapsed FL.<sup>51</sup> Therefore, at this time all patients with FL should be considered for entry into clinical trials to define the best regimens and to allow evaluation of the multitude of promising new drugs and antibodies that are now available. Patients who are ineligible for trials or who decline enrollment should receive individualized treatment. Patients with localized stage I or II disease may be offered local radiotherapy, observation, or chemoimmunotherapy. Elderly patients with asymptomatic stage III or IV disease may be best monitored with observation alone, particularly if their disease is of low volume and if they have multiple coexistent medical illnesses. Patients who are symptomatic, have cytopenias, massive splenomegaly, effusions, or bulky adenopathy should be treated with rituximab plus chemotherapy. Several regimens are acceptable

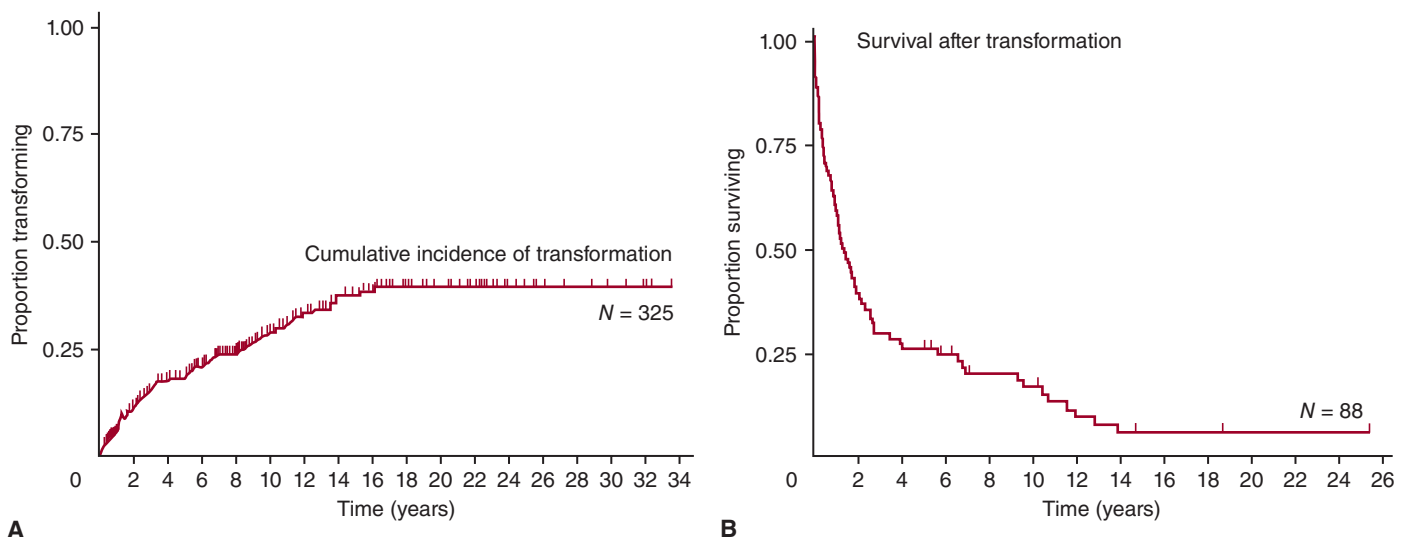


**Figure 10–6.** **A.** Transformation of follicular lymphoma (FL) to a histologic pattern compatible with rapid progression of disease. The line depicts the cumulative incidence of histologic transformation to a histologic pattern compatible with more rapid progression (e.g., diffuse large B-cell lymphoma) in 325 patients followed from the date of diagnosis of FL. **B.** The proportion of patients with FL surviving after transformation to a less-favorable histologic pattern. The graph shows the survival of the 88 patients from the date of their transformation to aggressive lymphoma. (Reproduced with permission from Montoto S, Davies AJ, Matthews J, et al: Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J Clin Oncol* 2007 Jun 10;25(17):2426–2433.)

including BR, R-CVP, and R-CHOP, with the last regimen being most appropriate for young patients with aggressive presentations and rapidly growing bulky adenopathy and B symptoms or for patients with grade 3 FL. The roles of maintenance rituximab and consolidative RIT following initial induction chemoimmunotherapy of newly diagnosed patients are contentious. It appears that either rituximab maintenance for 2 years or a single dose of consolidative RIT with  $^{90}\text{Y}$ -ibritumomab tiuxetan can prolong initial remission duration and PFS, but neither improves OS. Obinutuzumab may improve PFS when substituted for rituximab, but improved OS has not been demonstrated to date and toxicity may be modestly increased. Management of FL following relapse depends on the patient's initial treatment and the resultant remission duration. If the first remission lasts many years, the initial treatment regimen may again be used (except for anthracycline-containing regimens). If the initial remission is short, an alternative second-line regimen should be selected from among the many available options, including BR, R-CVP, R-CHOP, R-FND (rituximab, fludarabine, mitoxantrone [Novantrone], and dexamethasone), and RIT. Since cases of early progression (within 2 years of initial chemoimmunotherapy) have less favorable outcomes in the long term, these individuals might benefit from novel or more aggressive approaches.<sup>82a</sup> In the near future, many additional attractive options will be available, including ibrutinib,<sup>83,84</sup> PI3K inhibitors,<sup>65a,85,86</sup> ABT-199,<sup>87,88</sup> antibody-drug conjugates,<sup>89</sup> and adoptive immunotherapy with chimeric antigen receptor modified T lymphocytes.<sup>90,91</sup> Patients with a good performance status who experience very short response durations should be considered for autologous or allogeneic stem cell transplantation. Patients who undergo histologic transformation should receive R-CHOP and be considered for stem cell transplantation in some cases.

## COURSE AND PROGNOSIS

FL has been considered an indolent but incurable disease for which the median survival of approximately 10 years is minimally affected by medical interventions. This attitude is no longer valid, and survival of FL patients has been progressively increasing over the past 20 years (Fig. 10-7).<sup>92-94</sup> Much of the improvement in survival appears attributable to the introduction of rituximab, better salvage therapies (bendamustine, RIT), improved supportive care measures, and the wider implementation of stem cell transplantation. Controversy persists over whether any of the grades of FL are curable with standard chemoimmunotherapy regimens, although it is clear that OS has improved

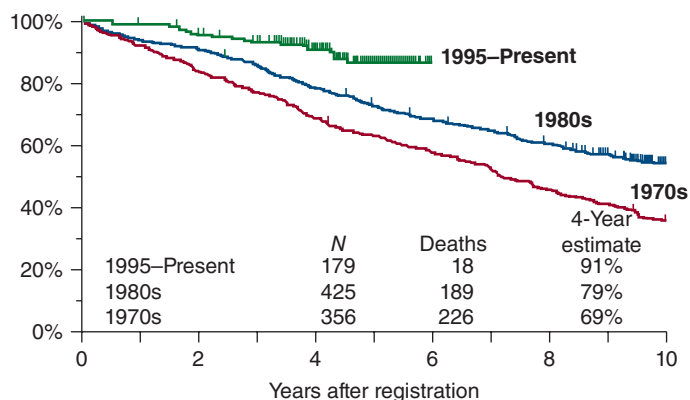


**Figure 10-7.** Improved survival of patients with follicular lymphoma treated by the Southwest Oncology Group. (Reproduced with permission from Fisher RI, LeBlanc M, Press OW, et al: New treatment options have changed the survival of patients with follicular lymphoma. *J Clin Oncol* 2005 Nov 20;23(33):8447-8452.)

significantly in the last decade. Further clinical research will settle these disputes and permit formulation of a consensus standard of care for the management of both newly diagnosed and relapsed FL.

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# CHAPTER 11

## MANTLE CELL LYMPHOMA

Martin Dreyling

### SUMMARY

Mantle cell lymphoma is a distinct subtype of non-Hodgkin lymphoma with a pathognomonic chromosomal translocation t(11;14), leading to constitutive cyclin D1 overexpression. The clinical presentation usually is characterized by widespread disease, occurring more often in male patients older than age 60 years. Despite high initial response rates, early relapses occur frequently after conventional chemotherapy, resulting in a median survival of only 3 to 5 years. However, 10 to 15 percent of patients present with a more indolent, chronic course. Dose-intensified treatment regimens containing cytarabine, rituximab, and autologous stem cell transplantation can achieve long-term remissions in patients fit enough to tolerate such aggressive therapy. For the majority of elderly patients, rituximab maintenance therapy can result in prolonged survival. Targeted approaches, including proteasome inhibitors, immunomodulatory drugs, and inhibitors of the B-cell receptor pathway, have proven highly efficacious in patients with relapsed disease and should be implemented in a multimodal treatment plan.

### DEFINITION AND HISTOLOGY

Mantle cell lymphoma (MCL) was originally named centrocytic lymphoma or subsumed under the term intermediate lymphocytic lymphoma. In 1992, the term *mantle cell lymphoma* was adopted for this entity because of morphologic and immunophenotypic similarities of the malignant cells to lymphocytes of the mantle zone of germinal centers.<sup>1</sup> In 1994, the term *mantle cell lymphoma* was incorporated into the revised European-American classification of the International Lymphoma Study Group and remains a distinctive lymphoma subtype in the World Health Organization classification of malignant lymphoid disorders.<sup>2,3</sup>

**Acronyms and Abbreviations:** ASCT, autologous stem cell transplantation; ATM, ataxia-telangiectasia mutant; BR, bendamustine and rituximab; BTK, Bruton tyrosine kinase; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CLL, chronic lymphocytic leukemia; DHAP, dexamethasone, high-dose cytarabine, and cisplatin; E2F, elongation factor 2; LDH, lactate dehydrogenase; MCL, mantle cell lymphoma; MIPI, Mantle Cell Lymphoma International Prognostic Index; mTOR, mammalian target of rapamycin; MTX, methotrexate; NF- $\kappa$ B, nuclear factor kappa B; PI3K, phosphoinositol 3'-kinase; PFS, progression-free survival; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CVP, rituximab, cyclophosphamide, vincristine, and prednisone; R-hyperCVAD, rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; slg, secretory immunoglobulin; TBI, total-body irradiation.

Based on cytology, the classical form is characterized by small-to-intermediate size cells with irregular, cleaved nuclei, dense chromatin, and indistinct nucleoli. A *leukemic nonnodal* variant, resembling chronic lymphocytic leukemia (CLL), associated with missing SOX-11 expression, may be associated with a more indolent course.<sup>3,4</sup> In contrast, the blastoid cell variant, including a blastic and a pleomorphic phenotype, displays a more aggressive clinical course.<sup>3</sup>

Histologically, MCL most frequently displays a diffuse infiltration of the lymph nodes, less commonly a nodular pattern, and, rarely, a mantle zone pattern, the last of which may represent an earlier phase of the disease.<sup>5</sup> The immunophenotype of the cells resembles the lymphocytes in the mantle zone of normal germinal follicles and is characterized by coexpression of B-cell antigens (CD19+, CD20+, CD22+, CD43+, CD79+, secretory immunoglobulin [slg] M+, sIgD+), and the T-cell-associated marker CD5+. Based on their predominantly pregerminal center origin, MCL cells stain strongly for the antiapoptotic protein BCL-2 but are negative for germinal center markers like CD10 and BCL-6.<sup>3</sup>

Because of the morphologic heterogeneity of MCL, detection of the MCL genetic hallmark, either by immunohistochemistry (cyclin D1 overexpression) or fluorescence *in situ* hybridization (chromosomal translocation t[11;14](q13;q32)) is crucial to confirm the diagnosis. In rare cases that are negative for cyclin D1, staining for SOX11, a transcription factor specifically expressed in more than 90 percent of MCL cases, may help to establish the diagnosis.<sup>6</sup>

### EPIDEMIOLOGY

MCL represents approximately 6 percent of all non-Hodgkin lymphomas, although a lower incidence has also been reported.<sup>7,8</sup> Between 1992 and 2004, the age-adjusted annual incidence more than doubled, from about 0.3 to 0.7 cases per 100,000. The incidence is more than twice as high in males and increases with age. Median age at presentation is approximately 65 years. No specific etiologic agent has been associated with MCL.

### ETIOLOGY AND PATHOGENESIS

The chromosomal translocation t(11;14) results in overexpression of cyclin D1, a cell cycle protein not normally expressed in lymphoid cells. The very rare cyclin D1-negative cases usually overexpress cyclin D2 or D3.<sup>6</sup> The cytogenetic abnormality t(11;14) is the primary event in the pathogenesis of MCL, facilitating the deregulation of the cell cycle at the G<sub>1</sub>-S phase transition.<sup>9</sup> However, additional genetic events are required for the clinical manifestation of MCL. Thus, a low copy number of the t(11;14) translocation has been found in the blood cells of 1 to 2 percent of healthy individuals without evidence of clinical disease.<sup>10</sup> Cytogenetic studies have identified frequent secondary genetic alterations that are involved in cell cycle dysregulation and DNA repair, which may explain why MCL has one of the highest levels of genomic instability among the malignant lymphoid neoplasms. These genetic abnormalities include losses in chromosomes 1p13-p31, 2q13, 6q23-27, 8p21, 9p21, 10p14-15, 11q22-23, 13q11-13, 13q14-34, 17p13, and 22q12; gains in chromosomes 3q25, 4p12-13, 7p21-22, 8q21, 9q22, 10p11-12, 12q13, and 18q11q23; and high copy number amplifications of certain chromosomal regions.<sup>11</sup>

Figure 11-1 depicts a proposed scheme relating genetic derangements with specific MCL subtypes. Cell cycle dysregulation is a hallmark of MCL. The overexpressed cyclin D1 complexes with CDK4, which results in phosphorylation of the retinoblastoma gene *RB1* and

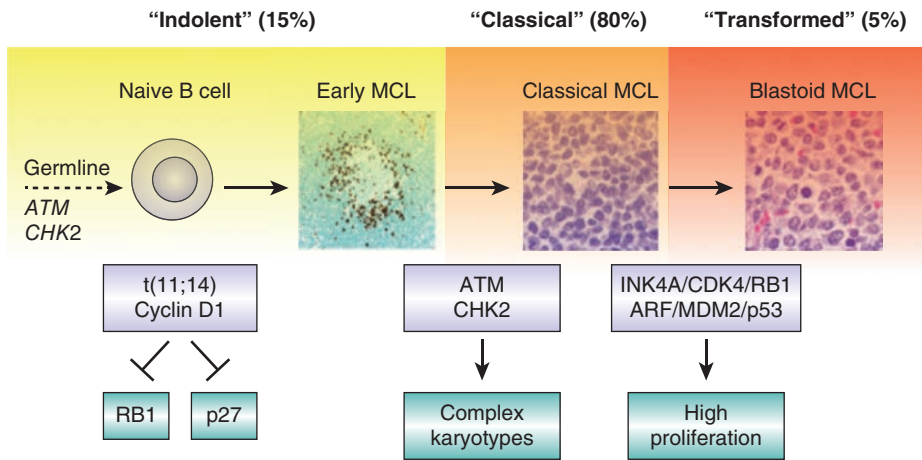


**Figure 11-1.** Proposed model of molecular pathogenesis of mantle cell lymphoma (MCL). The t(11;14) translocation leads to the constitutive deregulation of cyclin D1. Acquired inactivation of DNA damage response pathways may facilitate additional genetic alterations and the development of classical MCL. Further genetic alterations may target genes of the cell cycle and senescence regulatory pathways, leading to more proliferative and aggressive variants of MCL. ATM, ataxia-telangiectasia mutant; CDK, cyclin-dependent kinase; CHK, checkpoint kinase; INK, inhibitor of cyclin-dependent kinase; RB, retinoblastoma. (Modified with permission from Jares P, Colomer D, Campo E: Genetic and molecular pathogenesis of mantle cell lymphoma: Perspectives for new targeted therapeutics. *Nat Rev Cancer* 2007 Oct;7(10):750–762.)

release of elongation factor 2 (E2F) transcription factors and progression of the lymphoma cells into S phase. In addition, mutation of the ataxia-telangiectasia mutant (*ATM*) gene facilitates genomic instability in lymphoma cells through impaired response to DNA damage. Phosphoinositide 3'-kinase (PI3K) and mammalian target of rapamycin (mTOR) are important downstream targets of this signaling pathway. Finally, specific gene alterations, namely *p53* or *p16/CDKN2*, are associated with the blastoid variant and poor clinical outcome, whereas the absence of *SOX-11*, frequently associated with the leukemic, non-nodal variant, has been not confirmed to be an independent prognostic marker.<sup>12–13a</sup>

According to the characteristic cyclin D1 overexpression, molecular profiling has identified a *cell proliferation* gene signature that distinguishes patient subsets that differ by more than 5 years in median survival.<sup>14</sup> A five-gene model to predict survival in MCL based on formalin-fixed, paraffin-embedded tissue has been devised.<sup>15</sup> In the clinical setting, only Ki-67 expression, a cell cycle-related protein, as determined by immunohistochemistry, has been prospectively confirmed as a reliable prognostic marker that allows the identification of high-risk patients (Ki-67 >30 percent) who may qualify for more aggressive therapeutic approaches.<sup>5,16</sup> This marker is independent of clinical features and adds prognostic weight to the Mantle Cell Lymphoma International Prognostic Index (MIPI) score (see "Clinical Features and Risk Factors" section).

## CLINICAL FEATURES AND RISK FACTORS

MCL typically presents in an older male patient with lymphadenopathy in several sites (e.g., cervical, axillary, inguinal). The patient may be asymptomatic, but some experience fever, night sweats, or weight loss (Table 11-1).<sup>5,17</sup> The spleen is enlarged in 40 percent of patients. Marrow is involved with MCL in the vast majority of patients, and 50 percent of patients present with blood involvement, sometimes with an overt leukemic phase.

In 25 percent of cases, there is symptomatic gastrointestinal involvement, typically presenting as *polyposis coli*.<sup>18</sup> Gastrointestinal symptoms may include abdominal pain and diarrhea, signs of small bowel obstruction, or hematochezia. The intestinal polyps usually appear in the ileocecal region, and histopathologic analysis and immunocytochemistry are required to confirm the diagnosis of MCL involvement. Asymptomatic gastrointestinal involvement may be detected in

**TABLE 11-1.** Patient Characteristics at Presentation (304 Cases)

| Characteristic                     | Number |
|------------------------------------|--------|
| Age (years)                        |        |
| <60                                | 123    |
| >60                                | 178    |
| Sex                                |        |
| Male                               | 230    |
| Female                             | 71     |
| Stage                              |        |
| I–II                               | 23     |
| III–IV                             | 267    |
| Status (World Health Organization) |        |
| 0–1                                | 233    |
| ≥2                                 | 43     |
| Lactate dehydrogenase              |        |
| Elevated                           | 56     |
| Normal                             | 140    |
| International Prognostic Index     |        |
| 0–1                                | 15     |
| ≥2                                 | 75     |
| Marrow involvement                 |        |
| Yes                                | 207    |
| No                                 | 81     |
| B symptoms                         |        |
| Yes                                | 107    |
| No                                 | 155    |
| Extranodal involvement             |        |
| Yes                                | 161    |
| No                                 | 16     |

Data from Tiemann M, Schrader C, Klapper W, et al: European MCL Network: Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): A clinicopathological study from the European MCL Network. *Br J Haematol* 2005 Oct;131(1):29–38.



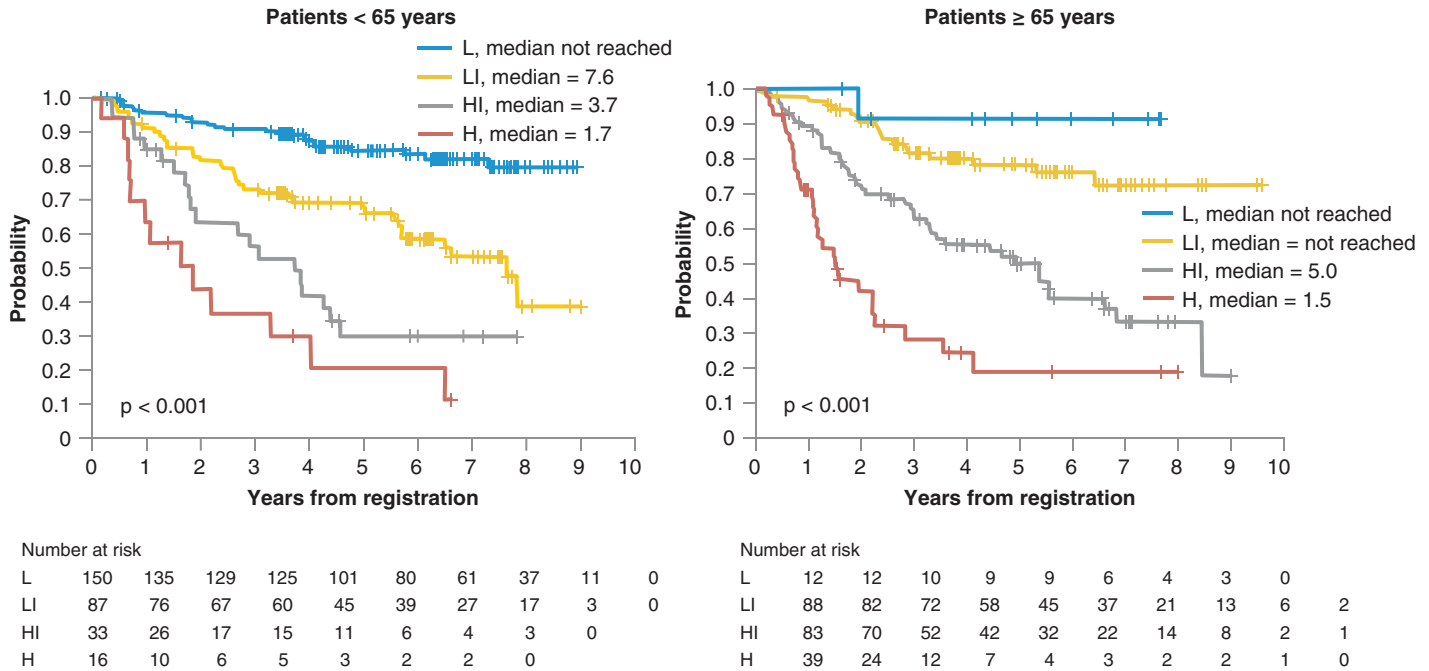


**Figure 11-2.** Overall survival according to combined Mantle Cell Lymphoma International Prognostic Index (MIPI-c). L, low risk; LI, intermediate risk; HI, high intermediate risk; H, high risk. (Reproduced with permission from Hoster E, Rosenwald A, Berger F, et al: Prognostic Value of Ki-67 Index, Cytology, and Growth Pattern in Mantle-Cell Lymphoma: Results From Randomized Trials of the European Mantle Cell Lymphoma Network. *J Clin Oncol* 2016; 34(12):1386–1389.)

up to 90 percent of cases; thus, endoscopy and histologic analysis of random biopsies are recommended, especially in the few cases with localized stage.

The frequency of CNS disease is low at first diagnosis but increases with subsequent relapses and correlates with elevated lactate dehydrogenase (LDH), blastoid cytology, and cell proliferation (Ki-67).<sup>19</sup> There is no consensus on the need for CNS prophylaxis.

Prognostic parameters include the serum level of  $\beta_2$ -microglobulin and LDH, blastoid cytology, age, Ann Arbor stage, extranodal presentation, and constitutional symptoms, among others.<sup>5</sup> Most of these prognostic variables were determined retrospectively and are based on doxorubicin-containing regimens only. Thus, a prognostic model, the MIPI, was established that implements four independent prognostic factors: age, performance status, LDH, and leukocyte count (Fig. 11-2).<sup>20</sup> This score has been confirmed in numerous series, including a combined score incorporating cell proliferation (MIPI-c) corroborating the reliability of this score for various chemotherapy-based regimens.<sup>20,21</sup>

Based on frequent marrow involvement, detection of minimal residual disease with patient-specific primers allows the detection of one malignant cell in  $10^5$  to  $10^6$  background cells. A number of reports have confirmed a correlation between molecular residual disease and clinical recurrence.<sup>22,23</sup> This fact has been used in trials evaluating the value of preemptive treatment with rituximab prior to emergence of clinical evidence of recurrence.

## DIFFERENTIAL DIAGNOSIS

The clinical presentation of MCL may resemble CLL or other indolent nodal lymphomas. The immunophenotype of CLL is similar, with coexpression of immunoglobulin (Ig) M and IgD and of the B-cell-associated antigens CD19 and CD20 and aberrant expression of the T-cell antigen CD5. In contrast to MCL cells, however, CD23 is typically highly expressed in CLL. Like follicular lymphoma, MCL is positive for CD20 and BCL-2, but in contrast to follicular lymphoma, MCL is negative for

CD10 and BCL-6. However, because these expression patterns vary, analysis of cyclin D1 overexpression or t(11;14) remains crucial to confirm or exclude the diagnosis of MCL.

## THERAPY

In general, MCL is still considered incurable, and most patients follow an aggressive clinical course. However, 10 to 15 percent of patients may exhibit a more indolent evolution and may not require therapy for several years. These cases are commonly characterized by a leukemic non-nodal presentation with only marrow involvement and splenomegaly.<sup>4</sup> Sox-11 negativity may also identify some of these more indolent cases, although its prognostic role is overcome by additional *p53* mutations that may cause an aggressive clinical evolution (see Fig. 11-1).<sup>3,4</sup> Thus, the reliable diagnosis of indolent cases is difficult, and a short, watchful, waiting period under close observation seems to be appropriate in cases with low tumor burden.<sup>24</sup>

## LOCALIZED STAGE

Localized disease with low tumor burden is rare. A small retrospective review has suggested long-term remissions after involved-field radiotherapy (30 to 36 Gy).<sup>25</sup> In contrast, in a randomized trial, all patients relapsed within a year.<sup>26</sup> Thus, a shortened chemotherapy induction followed by radiation consolidation seems to be most appropriate.

## ADVANCED STAGE

### Conventional Chemotherapy

Because of the typical aggressive clinical presentation, anthracycline-containing chemotherapy has usually been applied to MCL in the past, although a small randomized trial did not confirm a major clinical benefit.<sup>27</sup> Complete remission rates are only 30 to 40 percent with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), typically with a short duration of response of 10 to 12 months.<sup>28</sup>

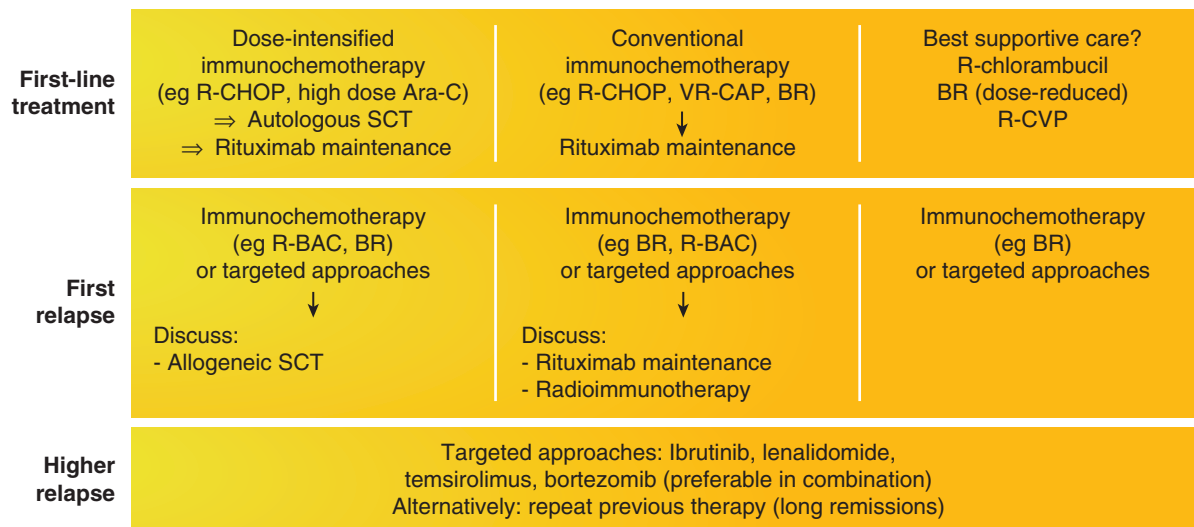


**Figure 11-3.** Clinical recommendations for mantle cell lymphoma outside of studies. Ara-C, cytarabine; BAC, bendamustine, cytarabine; BR, bendamustine, rituximab; CAP, cyclophosphamide, doxorubicin, prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CVP, cyclophosphamide, vincristine, prednisone; R, rituximab; SCT, stem cell transplant; VR, bortezomib, rituximab. (Modified with permission from Dreyling M: *European Mantle Cell Lymphoma Network: Mantle cell lymphoma: biology, clinical presentation, and therapeutic approaches*. Am Soc Clin Oncol Educ Book 2014;191-198.)

### Combined Immunochemotherapy

Rituximab monotherapy achieves low response rates of approximately 25 percent and should be used only in medically unfit patients who are not able to tolerate cytotoxic therapy (Fig. 11-3).<sup>29</sup> On the other hand, addition of rituximab to chemotherapy has been shown to significantly improve complete response rates, overall response rates, and overall survival in a systematic meta-analysis, making immunochemotherapy the standard of care in both first-line and relapsed settings for patients with advanced-stage MCL (Table 11-2).<sup>30-41</sup> In patients with an overt leukemic phase and high lymphocyte counts, the first dose of rituximab should be delivered with caution because of the risk of tumor lysis syndrome or cytokine-release syndrome.

In a randomized trial, rituximab with CHOP considerably improved response rates (94 percent vs. 75 percent) and time to treatment failure in comparison to CHOP chemotherapy alone.<sup>31</sup> In contrast, rituximab with cyclophosphamide, vincristine, and prednisone (R-CVP) resulted in significantly inferior response rates and progression-free survival (PFS) and cannot be recommended in patients with MCL.<sup>41</sup> Similarly, fludarabine combinations resulted in prolonged cytopenias and in overall survival rates significantly inferior (46 percent vs. 62 percent at 4 years) to those achieved with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in a large prospective European trial.<sup>36</sup> In contrast, a bendamustine-based combination resulted in similar response rates (93 percent vs. 91 percent) and PFS in two randomized trials but a more favorable toxicity profile, particularly with regard to alopecia and peripheral neuropathy, making the bendamustine and rituximab (BR) regimen the most commonly administered regimen currently, especially in elderly patients.<sup>38,41</sup> Finally, an intensified approach combining bendamustine with cytarabine resulted in impressive response rates (100 percent), but significant thrombocytopenia, suggesting that this regimen should be used only in very fit patients.<sup>39</sup>

In conclusion, BR and R-CHOP represent the current standard approaches in older patients, who represent the majority of MCL

patients (see Fig. 11-2). Based on clinical presentation, BR may be preferable, especially in patients with CLL-like presentation, whereas CHOP seems to be appropriate in the more aggressive cases with elevated LDH. Especially in blastoid variants, one might consider cytarabine-containing regimens based on the improved results in younger patients.<sup>42</sup> However, such an individualized approach has never been tested in a prospective fashion.

### Maintenance/Consolidation

The concept of regular rituximab maintenance, previously established in follicular lymphoma, has also been investigated in MCL.<sup>36</sup> After conventional R-CHOP induction, continuous antibody maintenance results in an impressive prolongation of both PFS (58 percent vs. 29 percent at 4 years) and overall survival (79 percent vs. 67 percent at 4 years) so that rituximab maintenance is now generally recommended.

Alternatively, radioimmunotherapy consolidation has been tested after a shortened R-CHOP induction. Again, survival rates seem to be prolonged with a median time to treatment failure of 31 months, but results seem to be inferior to ongoing rituximab maintenance, possibly because of the single-application scheme used with radioimmunotherapy.<sup>37</sup>

### Intensive Chemotherapy with and without Stem Cell Transplantation

In several studies, either intensified upfront therapy or the addition of high-dose consolidation followed by autologous stem cell transplantation (ASCT) resulted in impressive survival rates (Table 11-3).<sup>42-56</sup> A randomized trial has proven that ASCT prolongs PFS in comparison to CHOP-like induction therapy alone (3.3 vs. 1.4 years).<sup>43</sup> In a subsequent meta-analysis, overall survival was also significantly prolonged by ASCT, independent of the addition of rituximab.<sup>54</sup> *In vivo* purging with a rituximab-containing induction regimen was shown to further improve long-term survival. Various studies have investigated the potential benefit of cytarabine-containing induction regimens (Table 11-3). Most important, a randomized trial demonstrated that the median PFS was almost

**TABLE 11–2.** Conventional Immunochemotherapy (+/– Targeted Approaches) for Mantle Cell Lymphoma

| Author (Year)                       | Phase | Number of Patients | Regimen                                                       | ORR% (CR%) | Median PFS (Months)                | 2-Year OS (%)                |
|-------------------------------------|-------|--------------------|---------------------------------------------------------------|------------|------------------------------------|------------------------------|
| Howard (2002) <sup>30</sup>         | II    | 40                 | R-CHOP                                                        | 96 (48)    | 17                                 | 95 (3 years)                 |
| Lenz (2005) <sup>31</sup>           | III   | 112                | CHOP                                                          | 75 (7)     | 14 (TTF)                           | 77                           |
|                                     |       |                    | R-CHOP                                                        | 94 (34)    | 21 (TTF)                           | 77                           |
| Herold (2008) <sup>32</sup>         | III   | 90                 | MCP                                                           | 63 (15)    | 18                                 | 52 (4 years)                 |
|                                     |       |                    | R-MCP                                                         | 71 (32)    | 20                                 | 55 (4 years)                 |
| Gressin (2010) <sup>34</sup>        | II    | 113                | R-VAD-C                                                       | 73 (48)    | 18 (no ASCT)<br>58 (ASCT)          | 62 (3 years)                 |
| Sachanas (2011) <sup>35</sup>       | II    | 20                 | R-chlorambucil                                                | 95 (90)    | 89% (3 years)                      | 95 (3 years)                 |
| Kenkre (2011) <sup>33</sup>         | II    | 22                 | R-hyperCVAD<br>R maintenance                                  | 77 (64)    | 38                                 | 62 (4 years)                 |
| Kluin-Nelemans (2012) <sup>36</sup> | III   | 485                | R-CHOP                                                        | 86 (34)    | 28 (TTF)                           | 62 (4 years)                 |
|                                     |       |                    | R-FC                                                          | 78 (40)    | 26 (TTF)                           | 47 (4 years)                 |
|                                     |       |                    | I maintenance<br>R maintenance                                | NA         | 29% (4 year DR)<br>58% (4 year DR) | 67 (4 years)<br>79 (4 years) |
| Smith (2012) <sup>37</sup>          | II    | 50                 | R-CHOP<br><sup>90</sup> Y-Ibritumumab                         | 64 (46)    | 31 (TTF)                           | 73 (5 years)                 |
| Rummel (2013) <sup>38</sup>         | III   | 94                 | R-CHOP                                                        | 91 (30)    | 21                                 | No difference                |
|                                     |       |                    | BR                                                            | 93 (40)    | 35                                 |                              |
| Visco (2013) <sup>39</sup>          | II    | 20                 | R-BAC                                                         | 100 (95)   | 95% (2 years)                      | 93                           |
| Ruan (2011) <sup>93</sup>           | II    | 35                 | R-CHOP + bortezomib                                           | 91 (72)    | 44% (2 years)                      | 86                           |
| Houot (2012) <sup>99</sup>          | II    | 29                 | R-doxorubicin/<br>dexamethasone/<br>chlorambucil + bortezomib | 79 (59)    | 26                                 | 69                           |
| Chang (2014) <sup>57</sup>          | II    | 75                 | R-hyperCVAD + bortezomib<br>R maintenance                     | 95 (68)    | 67% (3 years)                      | 91 (3 years)                 |
| Robak (2015) <sup>98</sup>          | III   | 487                | R-CHOP                                                        | 89 (53)    | 14                                 | 54 (4 years)                 |
|                                     |       |                    | R-CHP + bortezomib                                            | 92 (42)    | 25                                 | 64 (4 years)                 |
| Inwards (2014) <sup>101</sup>       | I     | 17                 | R-cladribine + temsirolimus                                   | 94 (53)    | 19                                 | 65                           |

ASCT, autologous stem cell transplantation; BR, bendamustine and rituximab; CR, complete response; DR, duration of remission; MCP, mitoxantrone, chlorambucil, and prednisolone; NA, not available; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R-BAC, rituximab, bendamustine, and cytarabine; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CHP, rituximab, cyclophosphamide, doxorubicin, and prednisone; R-FC, rituximab, fludarabine, and cyclophosphamide; R-hyperCVAD, rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; R-MCP, rituximab, mitoxantrone, chlorambucil, and prednisone; TTF, time to treatment failure; <sup>90</sup>Y, yttrium-90.

The upper panel includes ten studies using immunochemotherapy; the lower panel includes five studies that combine rituximab with targeted drugs.

doubled by the administration of the R-CHOP/DHAP (dexamethasone, high-dose cytarabine, and cisplatin) regimen (7.6 vs. 3.8 years) compared to administration of R-CHOP alone prior to ASCT.<sup>42</sup> In contrast, a phase II study suggested that high-dose methotrexate (MTX) adds significant organ toxicity, but similar long-term remissions could also be achieved with considerably reduced doses.<sup>44</sup>

A study-to-study comparison suggested a benefit of a total-body irradiation (TBI)-containing high-dose consolidation in patients with only partial remission after induction, whereas such benefit was not observed for the addition of conventionally dosed radioimmunotherapy.<sup>55,56</sup> A

randomized trial has confirmed a benefit from subsequent rituximab maintenance, resulting in significantly improved PFS and overall survival rates using this approach.<sup>50</sup>

Alternatively, upfront dose intensification may be applied. The rituximab plus hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (R-hyperCVAD) regimen has achieved high complete response rates and long-term remissions in various trials.<sup>51–53</sup> However, this regimen is hampered by significant therapy-associated toxicity, including secondary malignancies, and should only be considered in young, fit patients.



**TABLE 11-3.** Dose-Intensified Immunochemotherapy of Mantle Cell Lymphoma

| Author (Year)                    | Phase | Number of Patients | Regimen                           | ORR% (CR%) | Median PFS (Years) | OS (%)         |
|----------------------------------|-------|--------------------|-----------------------------------|------------|--------------------|----------------|
| Dreyling (2005) <sup>43</sup>    | III   | 122                | R-CHOP + ASCT                     | 98 (81)    | 3.3                | 83 (3 years)   |
|                                  |       |                    | R-CHOP + IFN                      | 99 (37)    | 1.4                | 77 (3 years)   |
| de Guibert (2006) <sup>48</sup>  | II    | 17                 | R-DHAP + ASCT                     | 100 (94)   | 76% (3 years)      | 75% (3 years)  |
|                                  |       | 7                  | R-DHAP                            | 86 (86)    | NA                 | NA             |
| Damon (2009) <sup>44</sup>       | II    | 77                 | R-CHOP/MTX/Ara-C/etoposide + ASCT | 88 (69)    | 56% (5 years)      | 64 (5 years)   |
| van 't Veer (2009) <sup>45</sup> | II    | 87                 | R-CHOP/Ara-C + ASCT               | 70 (64)    | 36% (4 years)      | 56 (4 years)   |
| Magni (2009) <sup>49</sup>       | II    | 28                 | Sequential R-chemo + ASCT         | 100 (100)  | 57% (low risk)     | 76 (low risk)  |
|                                  |       |                    |                                   |            | 34% (high risk)    | 68 (high risk) |
| Eskelund (2016) <sup>46</sup>    | II    | 160                | R-maxiCHOP/Ara-C + ASCT           | 96 (54)    | 7.4                | 64 (10 years)  |
| Hermine (2016) <sup>42</sup>     | III   | 455                | R-CHOP + ASCT                     | 98 (63)    | 3.8                | 67 (5 years)   |
|                                  |       |                    | R-CHOP/DHAP + ASCT                | 99 (61)    | 7.3                | 74 (5 years)   |
| Delarue (2013) <sup>47</sup>     | II    | 60                 | R-CHOP/DHAP + ASCT                | 100 (96)   | 6.9                | 75 (5 years)   |
| Le Gouill (2016) <sup>50</sup>   | III   | 299                | R-DHAP + ASCT                     | NA         | 73% (3 years)      | 84% (3 years)  |
|                                  |       |                    | R-DHAP + ASCT + R maintenance     | NA         | 89% (3 years)      | 93% (3 years)  |
| Romaguera (2010) <sup>51</sup>   | II    | 97                 | R-hyperCVAD                       | 97 (87)    | 4.5                | 64 (10 years)  |
| Merli (2012) <sup>52</sup>       | II    | 60                 | R-hyperCVAD                       | 83 (72)    | 61% (5 years)      | 73 (5 years)   |
| Bernstein (2013) <sup>53</sup>   | II    | 2013               | R-hyperCVAD                       | 86 (55)    | 4.8                | 63 (5 years)   |

Ara-C, cytarabine; ASCT, autologous stem cell transplantation; CR, complete response; DHAP, dexamethasone, high-dose cytarabine, and cisplatin; IFN, interferon; MTX, methotrexate; NA, not available; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R, rituximab; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-DHAP, rituximab, dexamethasone, high-dose cytarabine, and cisplatin; R-hyperCVAD, rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone; R-maxiCHOP, cyclophosphamide, hydroxydaunorubicin, vincristine, and prednisone.

The first nine studies in the Table used sequential intensification with autologous stem cell transplantation (ASCT); the last three studies in the Table used upfront intensification.

## RECURRENT AND REFRACTORY DISEASE

### Salvage Chemotherapy

The inherent resistance of MCL to conventional doses of chemotherapy becomes especially apparent in relapsed disease. Conventional immunochemotherapy options, some of them highly effective in first-line treatment, achieve only short-term remissions in relapsed disease (Table 11-4).<sup>39,58-62</sup> Thus, consolidation with ASCT deserves consideration if not already employed in the frontline setting. Unfortunately, long-term results of this approach in recurrent/refractory MCL are rather sobering.

In younger patients responding to salvage therapy, it is recommended to discuss the option of potentially curative allogeneic transplantation, based on the observed graft-versus-lymphoma activity in MCL. Reduced-intensity conditioning may be applicable also in patients older than age 60 years but is hampered by delayed toxicities, including chronic graft-versus-host disease and 20 to 25 percent treatment-related mortality.<sup>63-66</sup>

In elderly patients, one might consider rituximab maintenance if not administered previously. Alternatively, radioimmunotherapy consolidation has achieved long-term remissions in some patients, although efficacy as a single approach is limited, with a median time to progression of only 5 months.<sup>67,68</sup>

### Targeted Approaches

Chemotherapy alone has only short-term activity in relapsed disease; therefore, a number of targeted approaches have been investigated,

especially in relapsed MCL, both as single agents and in combination with chemotherapy (Tables 11-4 and 11-5).<sup>69-99</sup>

**B-Cell Receptor Pathway** Small molecules targeting the B-cell receptor have achieved remarkable response rates in relapsed MCL. The Bruton tyrosine kinase (BTK) inhibitor ibrutinib exhibited a response rate of 68 percent in relapsed disease.<sup>82</sup> Combination with rituximab led to a response in all cases with low Ki-67, whereas in highly proliferating disease, this approach was only effective in half of the patients.<sup>84</sup> The compound is very well tolerated with only slight immunosuppression, bleeding, and atrial fibrillation being the predominant side effects. However, early relapses with a highly aggressive course have been observed.<sup>85</sup> Thus, combinations of ibrutinib with other compounds are currently being tested in studies.

Idelalisib achieves similar high response rates of 62 percent in MCL, although many of the remissions are short lived.<sup>86</sup>

**Immunomodulatory Drugs** Based on its oral formulation and its favorable tolerability, lenalidomide represents an attractive option, especially in the context of continuous treatment. After long-term efficacy was observed in multiple myeloma, various studies confirmed its benefit in relapsed MCL as well, with a response rate of 35 to 50 percent.<sup>73-76</sup> In a randomized phase II trial, this approach was superior to monochemotherapy (response rate 40 percent vs. 11 percent).<sup>76</sup> Other studies have investigated combining lenalidomide with chemotherapy but have suggested a high rate of infectious complications and secondary neoplasias.<sup>94</sup>

**TABLE 11-4.** Conventional Immunochemotherapy of Relapsed Mantle Cell Lymphoma

| Author (Year)                      | Phase         | Number of Patients | Regimen                  | ORR% (CR%) | Median PFS (Months) | Median OS (Months) |
|------------------------------------|---------------|--------------------|--------------------------|------------|---------------------|--------------------|
| Forstpointner (2004) <sup>58</sup> | III           | 24                 | FCM                      | 46 (0)     | 4                   | 11                 |
|                                    |               | 24                 | R-FCM                    | 58 (29)    | 8                   | 65% (2 years)      |
| Rummel (2005) <sup>59</sup>        | II            | 16                 | BR                       | 75 (50)    | 18                  | NA                 |
| Robinson (2008) <sup>60</sup>      | II            | 12                 | BR                       | 92 (42)    | 19                  | NA                 |
| Weide (2007) <sup>61</sup>         | II            | 18                 | BMR                      | 78 (33)    | 21                  | 60% (2 years)      |
| Gironella (2012) <sup>62</sup>     | II            | 28                 | R-GemOx                  | 79 (75)    | 18                  | 30                 |
| Visco (2013) <sup>39</sup>         | II            | 20                 | R-BAC                    | 80 (70)    | 87% (2 years)       | 93% (2 years)      |
| Weigert (2009) <sup>90</sup>       | Retrospective | 8                  | R-Ara-C + bortezomib     | 50 (25)    | 5                   | 16                 |
| Ruan (2010) <sup>93</sup>          | II            | 22                 | R-PEP-C + bortezomib     | 73 (32)    | 10                  | 45% (2 years)      |
| Kouroukis (2011) <sup>89</sup>     | II            | 25                 | Gemcitabine + bortezomib | 60 (11)    | 11                  | NA                 |
| Friedberg (2011) <sup>91</sup>     | II            | 7                  | BR + bortezomib          | 71 (NA)    | NA                  | NA                 |
| Gerecitano (2011) <sup>92</sup>    | II            | 10                 | R-CP + bortezomib        | 60 (50)    | NA                  | NA                 |
| Furtado (2015) <sup>88</sup>       | II            | 46                 |                          |            |                     |                    |
|                                    |               |                    | CHOP                     | 48 (22)    | 8                   | 12                 |
|                                    |               |                    | CHOP + bortezomib        | 83 (35)    | 17                  | 36                 |
| Ruan (2010) <sup>93</sup>          | II            | 22                 | R-PEP-C + thalidomide    | 73 (32)    | 10                  | 45% (2 years)      |
| Hess (2015) <sup>96</sup>          | I             | 11                 | BR + temsirolimus        | 91 (45)    | 22                  | 92% (19 months)    |

BMR, bendamustine, mitoxantrone, rituximab; BR, bendamustine and rituximab; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CR, complete response; FCM, fludarabine, cyclophosphamide, and mitoxantrone; NA, not available; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R-Ara-C, rituximab and cytarabine; R-BAC, rituximab, bendamustine, and cytarabine; R-CP, rituximab, cyclophosphamide, and prednisone; R-FCM, rituximab, fludarabine, cyclophosphamide, and mitoxantrone; R-GemOx, rituximab, gemcitabine, and oxaliplatin; R-PEP-C, rituximab, prednisone, etoposide, procarbazine, and cyclophosphamide.

The first six studies in the Table used immunochemotherapy; the next six studies included bortezomib with chemotherapy or immunochemotherapy; the last two studies added either thalidomide or temsirolimus to immunochemotherapy.

**Proteasome Inhibitors** Bortezomib, a first-generation proteasome inhibitor, is thought to exert its effect in part through alterations in the nuclear factor kappa B (NF- $\kappa$ B). In relapsed disease, this single agent has shown response rates of 30 to 40 percent, with a median PFS of approximately 6 months, which led to the first Food and Drug Administration approval of a targeted drug in relapsed MCL.<sup>69-71</sup> In subsequent trials, bortezomib was evaluated in combination with different chemotherapy regimens to further improve remission rates and avoid cumulative side effects (Table 11-4).<sup>88-93</sup> Based on the encouraging data, a first-line trial has been completed. Substituting bortezomib for vincristine (within R-CHOP) led to an almost doubling of PFS.<sup>98</sup> In such a combined approach, bortezomib should preferably be administered only on days 1 and 4 of each cycle to avoid cumulative thrombocytopenia.

**Mammalian Target of Rapamycin Inhibitors** Temsirolimus has been registered by the European Union based on a randomized phase III trial proving its superiority compared to monotherapy in a highly refractory patient population (response rate, 22 percent vs. 2 percent).<sup>79</sup> Combining temsirolimus with chemotherapy resulted in objective responses in all evaluable patients in a phase I trial.<sup>96</sup>

## COURSE AND PROGNOSIS

The prospects for patients with MCL have significantly improved over the last few years based on advances in therapy, including the addition of rituximab and optimization of chemotherapy regimens, according to both tolerability (bendamustine) and efficacy (cytarabine, autologous transplantation; Fig. 11-3).<sup>100</sup> Accordingly, overall survival has been observed to improve from a median of 2.7 years in the 1980s to 5.8 years in the 1990s.<sup>100</sup>

Improved diagnostic tools, which allow the detection of pathognomonic cyclin D1 overexpression, show that MCL represents a spectrum of disease (see Fig. 11-1). Accordingly, a few patients may be followed with watchful waiting, whereas others present with a highly proliferative disease insensitive to conventionally dosed chemotherapy. In the majority of cases, initial responses are followed by a continuous relapse pattern, although remission durations have significantly improved as a result of improved frontline therapeutic approaches. The implementation of targeted therapies with proven efficacy in relapsed disease represents the current challenge in treatment of MCL (see Fig. 11-3). Besides ibrutinib and possibly lenalidomide, combined approaches are

**TABLE 11–5.** Therapy for Relapsed Mantle Cell Lymphoma

| Author (Year)                 | Phase | Number of Patients | Regimen                      | ORR% (CR%)      | Median PFS (Months) | Median OS (Months) |
|-------------------------------|-------|--------------------|------------------------------|-----------------|---------------------|--------------------|
| Goy (2009) <sup>69</sup>      | II    | 141                | Bortezomib                   | 33 (8)          | 6.7                 | 23.5 months        |
| Baiocchi (2011) <sup>70</sup> | II    | 13                 | R-bortezomib                 | 29 (29)         | 2                   | NA                 |
| Lamm (2011) <sup>71</sup>     | II    | 16                 | R-bortezomib/dexamethasone   | 81 (44)         | 12                  | 39                 |
| Kaufmann (2004) <sup>72</sup> | II    | 16                 | R-thalidomide                | 81 (31)         | 20                  | 75% (3 years)      |
| Zinzani (2013) <sup>73</sup>  | II    | 57                 | Lenalidomide                 | 35 (12)         | 8.8                 | NR                 |
| Goy (2013) <sup>74</sup>      | II    | 134                | Lenalidomide                 | 28 (88)         | 4                   | 19                 |
| Trnjeny (2016) <sup>76</sup>  | II    | 170                | Lenalidomide                 | 46 (11)         | 8.7                 | 27.9               |
|                               |       | 84                 | monochemotherapy             | 23 (8)          | 5.2                 | 21.2               |
| Wang (2012) <sup>75</sup>     | II    | 44                 | R-lenalidomide               | 57 (36)         | 11.1                | 24.3               |
| Witzig (2005) <sup>77</sup>   | II    | 34                 | Temsirolimus 250 mg          | 38 (3)          | 7 (TTP)             | 12                 |
| Ansell (2008) <sup>78</sup>   | II    | 27                 | Temsirolimus 25 mg           | 41 (4)          | 6 (TTP)             | 14                 |
| Hess (2009) <sup>79</sup>     | III   | 162                | Temsirolimus 175/75 mg       | 22 (2)          | 4.8                 | 12.8               |
|                               |       |                    | Temsirolimus 175/25 mg       | 6 (0)           | 3.4                 | 10                 |
|                               |       |                    | chemotherapy                 | 2 (2)           | 1.9                 | 9.7                |
| Ansell (2011) <sup>80</sup>   | II    | 69                 | R-temsirolimus               | 59 (19)         | 9.7                 | 29.5               |
| Hess (2016) <sup>96</sup>     | I/II  | 32                 | Temsirolimus, bendamustine-R | 87 (8)          | 18                  | 36                 |
| Renner (2012) <sup>81</sup>   | II    | 35                 | Everolimus                   | 20 (6)          | 6                   | NA                 |
| Wang (2013) <sup>82</sup>     | II    | 111                | Ibrutinib                    | 68 (21)         | 13.9                | NA                 |
| Dreyling (2016) <sup>83</sup> | III   | 280                | Ibrutinib vs. temsirolimus   | 72 (19)         | 14.6                | NR                 |
|                               |       |                    |                              | 40 (1)          | 6.2                 | 21.3               |
| Wang (2014) <sup>84</sup>     | II    |                    | R-ibrutinib                  | 100 (low Ki-67) | 90% (1 year)        | 90% (1 year)       |
|                               |       |                    |                              | 50 (high Ki-67) | 13.6                | 13.6               |
| Kahl (2014) <sup>86</sup>     | I     | 16                 | Idelalisib                   | 62 (NA)         | 3 (RD)              | NA                 |
| Dauids (2017) <sup>87</sup>   | I     | 28                 | Abt-199 (Venetoclax)         | 75 (21)         | 14                  | 82% (1 year)       |

CR, complete response; DR, duration of remission; NA, not available; NR, not reached; ORR, overall response rate; OS, overall survival; PFS progression-free survival; R, rituximab; TTP, time to progression.

required to achieve prolonged remission durations. In addition, predictive markers for distinct targeted therapies are urgently needed for more individualized treatment strategies that will allow patients to achieve optimal outcomes.

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## CHAPTER 12

# MARGINAL ZONE B-CELL LYMPHOMAS

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### SUMMARY

Indolent B-cell lymphomas deriving from the marginal zone include three specific entities: extranodal marginal zone (or mucosa-associated lymphoid tissue) lymphoma (EMZL), splenic marginal zone lymphoma (SMZL), and nodal marginal zone lymphoma (NMZL). The clinical and molecular characteristics are distinctive for each of these entities, although some phenotypic and genetic features are overlapping. EMZL is the most common entity, arising at virtually any extranodal site, commonly associated with chronic antigenic stimulation either as a result of an external infection (e.g., *Helicobacter pylori* in the stomach) or an autoimmune disease (such as Sjögren syndrome or Hashimoto thyroiditis). SMZL accounts for approximately 20 percent of all marginal zone lymphomas, with patients typically presenting with an enlarged spleen and involvement of marrow and splenic hilar lymph nodes. NMZL is the least common entity, representing approximately 10 percent of all marginal zone lymphomas and typically presenting with lymph node–based disease without splenic or extranodal site involvement.

### ● INTRODUCTION AND CLASSIFICATION

Marginal zone lymphomas (MZLs) represent a heterogeneous group of indolent lymphoproliferative disorders originating from memory B lymphocytes, which are normally present in the marginal zone—that is, the outer part of the mantle zone—of the secondary lymphoid follicles. The spleen and the mucosa-associated lymphoid tissues (MALTs) are the most frequently involved anatomic compartments; lymph nodes may also be involved, albeit rarely. The 2016 revision of the *World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues* identifies three distinct subtypes of MZL based on the involved site, the clinical presentation and course of the disease, as well as the molecular profiles, namely, extranodal MZL of MALT type (also termed *extranodal marginal zone lymphoma* [EMZL]); splenic marginal zone

**Acronyms and Abbreviations:** *AKT1*, protein kinase B $\alpha$ ; *BCL6*, B-cell CLL/lymphoma 6 gene; *BCL10*, B-cell CLL/lymphoma 10 gene; BCR, B-cell receptor; BTK, Bruton tyrosine kinase; CD, cluster of differentiation; EMZL, extranodal marginal zone lymphoma; HCV, hepatitis C virus; Ig, immunoglobulin; *IGHV*, immunoglobulin heavy-chain variable region gene; IPSID, immunoproliferative small intestinal disease; LDH, lactate dehydrogenase; LPL, lymphoplasmacytic lymphoma; MALT, mucosa-associated lymphoid tissue; MZL, marginal zone lymphoma; NF- $\kappa$ B, nuclear factor kappa B; NMZL, nodal marginal zone lymphoma; *PAX5*, paired box gene 5; *PIM1*, protooncogene proteins pim; R-CHOP, rituximab, cyclophosphamide, doxorubicin (hydroxydaunomycin), vincristine (Oncovin), prednisone; R-CVP, rituximab, cyclophosphamide, prednisone; ROS, reactive oxygen species; SMZL, splenic marginal zone lymphoma; WHO, World Health Organization.

lymphoma (SMZL); and nodal marginal zone lymphoma (NMZL).<sup>1,1a</sup> In addition, three provisional entities are recognized by the current WHO classification: pediatric NMZL, splenic diffuse red pulp lymphoma, and hairy cell leukemia variant. The first is a separate variant of NMZL that mainly involves head and neck lymph nodes in male patients of pediatric age, with an indolent course and a remarkably better prognosis compared to NMZL. The last two represent subtypes of splenic lymphomas with features overlapping with those of MZL.<sup>1a</sup>

In adults, MZLs account for 5 to 17 percent of all non-Hodgkin lymphomas (NHLs); MALT lymphoma is the most frequent overall, being the third most frequent NHL and representing 7 to 8 percent of all B-cell neoplasms. It mostly affects middle-aged adults, at a median age of 60 years, with a slight female preponderance and often in association with chronic antigenic stimulation, either as a consequence of a chronic infection or an autoimmune disease. Gastric MALT lymphoma is by far the most common clinical entity, displaying considerable geographic variability, with a higher incidence in areas associated with a high incidence of *Helicobacter pylori* infection. SMZL and NMZL represent 20 percent and 10 percent of MZLs, respectively, accounting for less than 2 percent of all NHLs. The median ages at disease onset are 65 years for SMZL and between 50 and 60 years for NMZL.<sup>2,3</sup>

Each lymphoma subtype is presented and discussed separately in terms of epidemiology, etiology and pathogenesis, clinical presentation, and treatment strategies.

### ● EXTRANODAL MARGINAL ZONE LYMPHOMA

#### DEFINITION

EMZL or MALT lymphoma is an extranodal lymphoma that arises both in organs with an anatomically well-defined MALT, such as the gut, the nasopharynx, and the lung (in which MALT represents an acquired and specialized immunologic barrier associated with highly permeable mucosal sites in close contact with the external environment), and in sites that normally lack lymphoid tissue but have accumulated B cells in response to a chronic infection or an autoimmune process, such as the salivary glands, the ocular adnexa (orbits, conjunctiva and lacrimal glands), the skin, the thyroid, the genitourinary tract (bladder, prostate, kidney, uterus), and the breasts.

#### EPIDEMIOLOGY

MALT lymphoma accounts for at least 50 percent of primary gastric lymphomas, typically presenting in middle-aged adults, with a female predominance. Gastric MALT lymphoma is particularly common in the northeastern part of Italy, which is an area of high prevalence of *H. pylori* infection. In contrast, a specific subtype of small intestinal MALT lymphoma (termed *immunoproliferative small intestinal disease* [IPSID]) tends to be more frequent in the Middle East, on the Indian subcontinent, and in the Cape Region of South Africa.

#### ETIOLOGY AND PATHOGENESIS

Sustained antigenic stimulation, triggered either by a chronic infection or by an autoimmune process, induces an initially polyclonal B-cell proliferation, as well as the development of an inflammatory response, with the attraction of neutrophils, which release reactive oxygen species (ROS). ROS are genotoxic and may induce a wide range of genetic abnormalities. Moreover, the prolonged proliferation of B lymphocytes induced by a chronic lymphoproliferative stimulus may increase the risk of DNA damage (double-strand breaks and translocations) as a consequence of the genetic instability of B cells during somatic hypermutation



and class-switch recombination. Genetic abnormalities tend to involve those genes related to the activation of nuclear factor kappa B (NF- $\kappa$ B), a key transcription factor regulating the expression of several survival- and proliferation-related genes in B cells during immune responses. The constitutive activation of this signaling pathway results in uncontrolled B-cell proliferation and subsequent neoplastic transformation of the involved tissue.

The long-standing antigenic stimulation explains how lymphoid infiltrates—or an acquired MALT—may appear in extranodal sites normally lacking MALT. The MALT that organizes in proximity to an epithelium receives stimuli from the epithelium itself (as occurs in autoimmune disorders) or from antigens entering the lymphoid tissue through the epithelium or via the afferent lymphatics (as during exogenous infections).

Microbial species thought to be associated with MALT lymphoma include *H. pylori* and *Helicobacter heilmannii*, hepatitis C virus (HCV), *Campylobacter jejuni*, *Borrelia burgdorferi*, and *Chlamydia psittaci*. These entities have been found to be related with the development of MALT lymphoma of the stomach, IPSID, cutaneous MALT lymphoma, and orbital MALT lymphoma, respectively.<sup>4–7</sup> *H. pylori* has been documented as the etiologic agent in more than 90 percent of gastric MALT lymphomas, which arise from *H. pylori*-stimulated autoreactive B cells.<sup>8</sup> *H. pylori* can be demonstrated by histology, polymerase chain reaction, or urea breath test and can be isolated and cultured. *H. pylori* appears to be a fundamental factor for the development of the disease, and its elimination often induces a remission of the lymphoma by itself without any other intervention, thus fulfilling all four of Koch's postulates and establishing the microorganism as the main etiologic factor. However, because only a minority of patients with an overt *H. pylori* infection develop the disease, it is obvious that the pathogenesis of gastric EMZL also depends on other, largely unknown factors, perhaps related to the host, the environment, or the virulence of the infecting *H. pylori* strain.

It has also been demonstrated that viable *C. psittaci* can be found not only in monocytes and macrophages infiltrating orbital MALT lymphoma, but also in patients' blood and conjunctiva, raising the possibility of microbial eradication and disease remission by antibiotic therapy with drugs such as doxycycline.<sup>9</sup>

Autoreactive B cells found in autoimmune diseases, such as those infiltrating the thyroid in Hashimoto thyroiditis or the salivary glands in Sjögren syndrome, organize progressively into a well-developed MALT, mimicking lymphoproliferation and eventually evolving into an overt lymphoma. Patients with Sjögren syndrome have a 44-fold increased risk of developing lymphoma, and patients with Hashimoto thyroiditis have a 70-fold increased risk of thyroid lymphoma.<sup>10,11</sup>

## GENETIC ABERRATIONS

Recurrent genomic lesions, including chromosomal translocations and unbalanced aberrations, can be detected in MALT lymphomas in up to 40 percent of the cases, depending on the specific aberration. The t(11;18)(q21;q21) translocation is the most common structural chromosomal abnormality in MALT lymphoma, being demonstrated in 15 to 40 percent of the cases, especially in gastric and lung MALT lymphomas.<sup>12</sup> Other chromosomal translocations include t(14;18)(q32;q21), t(1;14)(p22;q32), and t(3;14)(p13;q32), all involving the immunoglobulin heavy chain variable region (*IGHV*) gene on chromosome 14 and displaying a specific anatomic distribution (Table 12–1).<sup>13</sup> The presence of any of these chromosomal alterations correlates with a lack of any further genetic instability or chromosomal imbalances.

Current knowledge of the genetic lesions seen in MALT lymphoma suggests a model of multistage development and progression from a preneoplastic lesion to overt lymphoma. The accumulation of genetic abnormalities is associated with a loss of dependency from antigenic stimulation (with subsequent antibiotic resistance) and with possible histologic transformation.

## CLINICAL FEATURES

EMZL mostly presents as Ann Arbor stage IE disease, which implies the sole involvement of the extranodal site of origin, with possible extension of the disease to tributary lymph nodes in close proximity to the affected organ. Marrow and peripheral lymph node infiltration is uncommon, observed in less than 20 percent of the cases at presentation. The stomach is the most commonly involved organ, accounting for approximately one-third of cases. Other typical presentations include the salivary glands, the orbits and the ocular adnexa, the thyroid, the lungs, the skin, the breasts, the liver, and other gastrointestinal sites apart from the stomach. Disseminated disease can be documented in up to 25 percent of patients with gastric MALT lymphoma, although those with nongastrointestinal extranodal disease exhibit disseminated lymphoma in nearly half of the cases.<sup>14</sup> Gastric MALT lymphoma is often multifocal, which may explain the high rate of relapse in the gastric stump after surgical excision. Synchronous involvement of gastrointestinal and extraintestinal sites is detected in approximately 10 percent of cases.

Systemic lymphoma-related symptoms are generally uncommon, and the clinical aspects and presenting symptoms generally correlate with the primary location of the disease.

Gastric MALT lymphoma presents with nonspecific dyspepsia, epigastric pain, and nausea. Chronic bleeding may become evident

**TABLE 12–1. Common Genetic Lesions in Mucosa-Associated Lymphoid Tissue Lymphoma**

|                | Lesion            | Genes              | Frequency | Sites                                     |
|----------------|-------------------|--------------------|-----------|-------------------------------------------|
| Translocations | t(11;18)(q21;q21) | <i>BIRC3-MALT1</i> | 15–40%    | Stomach, lung                             |
|                | t(14;18)(q32;q21) | <i>IGHV-MALT1</i>  | 20%       | Lung, skin, ocular adnexa, salivary gland |
|                | t(1;14)(p22;q32)  | <i>IGHV-BCL10</i>  | <5%       | Stomach, lung                             |
|                | t(3;14)(p13;q32)  | <i>IGHV-FOXP1</i>  | <5%       | Unclear                                   |
| Gains          | +3; +3q           |                    | 20–40%    | No differences in sites                   |
|                | +18; +18q         |                    | 20–40%    | No differences in sites                   |
| Losses         | –6q23             | <i>TNFAIP3</i>     | 15–30%    | No differences in sites                   |

*BCL-10*, B-cell CLL (chronic lymphocytic leukemia)/lymphoma 10 gene; *BIRC3*, baculoviral IAP (inhibitor of apoptosis protein) repeat-containing 3 gene; *FOXP1*, forkhead box P1 gene; *IGHV*, immunoglobulin heavy-chain variable region gene; *MALT1*, mucosa-associated lymphoid tissue translocation gene 1; *TNFAIP3*, tumor necrosis factor- $\alpha$ -induced protein 3 gene.

with progressively worsening iron-deficiency anemia. The *antrum* is the most frequently involved portion of the organ, although any part of the stomach can be affected: intragastric nodularities, enlarged rugal folds, thickened gastric walls, irregularly shaped superficial erosions, and shallow ulcers all represent macroscopic features of this lymphoma.

MALT lymphomas of the ocular adnexa most often arise in the orbit (40 percent of cases), with masses that cause progressive proptosis, periorbital edema, and abnormalities in ocular motility and vision. The conjunctiva is the site of origin in approximately 35 to 40 percent of the cases, with bilateral involvement observed in nearly 15 percent of patients. More rarely, the lymphoma originates from the lacrimal gland (10 percent of the cases) or the eyelid.

Cutaneous MALT lymphoma generally presents with papules, plaques, or nodules mainly involving the trunk and the upper limbs; the occurrence of multiple lesions affecting noncontiguous regions is, however, not infrequent. Lesions may show spontaneous remissions, but cutaneous relapses are the rule.

Immunoproliferative small intestinal disease, which is considered a special variant of intestinal MALT lymphoma, usually manifests with a severe and unremitting malabsorption. The lymphoma usually remains confined to the upper intestine and the regional lymph nodes, spreading beyond the abdomen only in advanced stages of the disease and upon transformation into high-grade lymphoma.

## MORPHOLOGY

The neoplastic lymphocytes tend to infiltrate the marginal zone around reactive B-cell follicles, external to an intact follicular mantle. Their cytoplasm is pale, and they display small- to medium-size and irregularly shaped nuclei, with dispersed chromatin and inconspicuous nucleoli, either resembling germinal center centrocytes or monocytoid elements. Scattered, centroblast-like large cells may be present, although never predominant, as well as mature plasma cells in up to a third of cases. Lymphoepithelial lesions are characterized by invasion or necrotic destruction of the glandular epithelium by infiltrating lymphoma cells; they are highly characteristic of MALT lymphoma, particularly gastric MALT lymphoma (Fig. 12-1), although they are not pathognomonic. Germinal centers may also be colonized by MZL cells, thus conferring a vaguely nodular or follicular pattern; lymphoma cells may undergo a blastic or plasma cell differentiation. Upon immunohistochemistry, cells are CD20+, CD79a+, CD21+, and CD35+, lacking CD5, CD23,

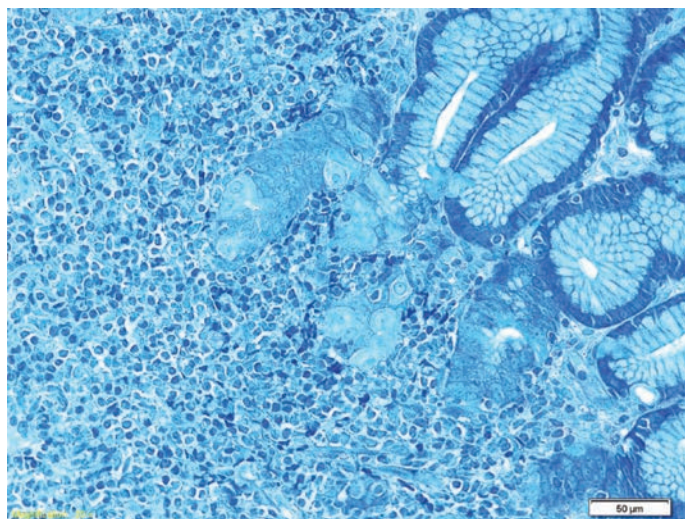


**Figure 12-1.** Gastric mucosa-associated lymphoid tissue lymphoma (Giemsa stain, magnification  $\times 200$ ). (Used with permission of Dr. Claudio Agostinelli.)

and CD10, typically expressing immunoglobulin (Ig) M (less often IgA or IgG), with an immunoglobulin light-chain restriction.

## DIFFERENTIAL DIAGNOSIS

A distinction between reactive (i.e., acquired) and neoplastic MALT is sometimes difficult, especially for MALT lymphoma at early stages of evolution. The demonstration of clonality by virtue of light-chain restriction (on immunohistochemistry or flow cytometry) is of great diagnostic value. Other entities to be considered are other indolent B-cell lymphomas involving extranodal sites, such as mantle cell lymphoma, small lymphocytic lymphoma, and follicular lymphoma. An extended immunohistochemical panel, including CD5, CD23, CD10, and cyclin D1, along with cytogenetic and molecular analysis is required to rule out other diagnoses.

## STAGING PROCEDURES

A complete and detailed patient's history and a full physical exam, along with blood studies evaluating renal and liver function, lactate dehydrogenase (LDH), serum protein electrophoresis and immunofixation, and complete serology for HIV, HCV, and hepatitis B virus are considered mandatory.<sup>15</sup> Cryoglobulins should be measured in HCV-positive individuals. Computed tomography of the neck, chest, abdomen, and pelvis, as well as marrow aspiration and biopsy, are required. *H. pylori* status should always be evaluated on the gastric biopsy or through a urea breath test and repeated after therapy if positive at baseline. Endoscopic ultrasound to evaluate the regional lymph nodes and gastric wall infiltration is also recommended in gastric MALT lymphoma. Fluorescence *in situ* hybridization for t(11;18) is optional but useful to guide therapy. Involvement with *C. jejuni*, *C. psittaci*, or *B. burgdorferi* may be detected in tumor biopsies from intestine, ocular adnexa, or skin, respectively; by polymerase chain reaction; by immunohistochemistry; or by *in situ* hybridization.

## THERAPY

*H. pylori* eradication therapy should be administered to all patients with *H. pylori*-positive MALT lymphomas, independent of stage at presentation or histologic grade. The outcome of *H. pylori* eradication must be confirmed by urea breath test at least 6 weeks after eradication therapy and at least 2 weeks after proton pump inhibitor withdrawal.

Antibiotic therapy is based on the epidemiology of the infection in the patient's country of residence and should take into account locally expected antibiotic resistance patterns. The most common approach is based on three drugs for 10 to 14 days: a proton pump inhibitor, in association with either amoxicillin or metronidazole and with clarithromycin.<sup>16</sup>

The role of surgery has been questioned, as gastric MALT lymphoma is generally multifocal, thus requiring an extensive (total or subtotal) gastrectomy, usually severely impairing the quality of life. Nevertheless, gastrectomy can be considered in cases with major hemorrhage, massive infiltration of the gastric walls (with an enhanced risk of perforation during chemotherapy), or pyloric stenosis.<sup>17</sup>

Patients with nongastric MALT lymphoma and gastric MALT lymphoma patients who fail to respond to *H. pylori* eradication or who have no evidence of *H. pylori* infection should be considered for alternative treatments. However, there is no evidence-based consensus delineating optimal alternative treatment strategies. Involved-field radiation therapy (25 to 35 Gy) is a reasonable option for localized disease.<sup>18,19</sup> Chemotherapy, rituximab immunotherapy, or a combination of both is effective in the treatment of all stages of disease (Table 12-2).<sup>20-26</sup> It is important to note that patients with the t(11;18) translocation are usually unresponsive to alkylating drugs if administered as single agents.

**TABLE 12-2.** Chemotherapy/Immunotherapy Experiences in Gastric Mucosa-Associated Lymphoid Tissue Lymphoma

| Study                    | No. of Patients | Early Stage | Treatment                        | Outcomes       |
|--------------------------|-----------------|-------------|----------------------------------|----------------|
| Hammel <sup>20</sup>     | 24              | 71%         | Cyclophosphamide or chlorambucil | 75% CR         |
| Avilés <sup>21</sup>     | 83              | 100%        | CHOP × 3 + CVP × 4               | 100% CR        |
| Jäger <sup>22</sup>      | 19              | 100%        | Cladribine                       | 100% CR        |
| Martinelli <sup>23</sup> | 27              | 86%         | Rituximab                        | 46% CR; 31% PR |
| Raderer <sup>24</sup>    | 7               | 57%         | R-CHOP/R-CNOP                    | 100% CR        |
| Salar <sup>25</sup>      | 21              | 64%         | Bendamustine + rituximab         | 94% CR; 6% PR  |
| Zucca <sup>29</sup>      | 53              | —           | Rituximab + chlorambucil         | 91% CR         |
|                          | 57              | —           | Chlorambucil                     | 61% CR         |
|                          | 61              | —           | Rituximab                        | 67% CR         |

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CNOP, cyclophosphamide, mitoxantrone, vincristine, prednisone; CR, complete response; CVP, cyclophosphamide, vincristine, prednisone; PR, partial response; R, rituximab.

Fludarabine has important antitumor activity,<sup>27</sup> especially when combined with rituximab.<sup>28</sup> The update of the International Extranodal Lymphoma Study Group trial in patients with MALT lymphoma has shown that rituximab combined with chlorambucil as first-line systemic treatment produces higher response rates, with better event-free and progression-free survival rates, than either chlorambucil or rituximab administered alone.<sup>29</sup> Patients with primary gastric localization, in particular, have shown better outcomes than did those with extragastric involvement. Radioimmunotherapy with <sup>90</sup>Y-ibritumomab tiuxetan is also effective for patients with MALT lymphoma.<sup>30</sup>

## COURSE AND PROGNOSIS

MALT lymphoma usually has a favorable outcome, with a 5-year overall survival greater than 85 percent in most series. The reported median time to progression seems to be more favorable for gastrointestinal MALT lymphomas than nongastrointestinal lymphomas; however, no significant differences in overall survival have been demonstrated between the two disease subgroups. Approximately 30 to 50 percent of patients with a *H. pylori*-positive gastric MALT lymphoma show persistent or progressive disease after *H. pylori* eradication with antibiotic therapy. Among complete responders, almost 15 percent relapse within 3 years, suggesting that additional therapies are required in a significant percentage of patients. Histologic transformation to diffuse large B-cell lymphoma has been reported in approximately 10 percent of the cases, usually as a late event that occurs independently from dissemination.

## ● SPLENIC MARGINAL ZONE LYMPHOMA

### DEFINITION AND EPIDEMIOLOGY

SMZL is a mature B-cell neoplasm arising from postgerminal center lymphocytes and involving the white pulp follicles of the spleen, the splenic hilar lymph nodes, the marrow and often the blood, showing characteristic neoplastic lymphoid elements referred to as “villous lymphocytes.” It accounts for 1 to 2 percent of all lymphomas, with a median age at diagnosis of nearly 65 years (range: 30 to 90 years) and a slight male predominance.

### ETIOLOGY AND PATHOGENESIS

The precise pathogenesis of SMZL is unknown: The cell of origin is a marginal zone B cell, which is believed to be of postgerminal center origin, as demonstrated by the presence of somatic hypermutations in *IGHV* genes<sup>31,32</sup>; however, up to one-third of cases are nonmutated. Conversely,

SMZL exhibits a low frequency of somatic mutations involving oncogenes such as *BCL-6*, *PAX5*, and *PIM1*, which suggests a pathway of differentiation that may not involve a transit through the germinal center.<sup>33</sup>

In cases of SMZL associated with chronic HCV infection, long-standing antigenic stimulation of B lymphocytes as a result of the interaction of the viral E2 glycoprotein with CD81 on B cells is responsible for lymphocyte activation through the B-cell receptor (BCR), which ultimately leads to an increased rate of proliferation of B cells. Antiviral treatments can induce remissions of SMZL, thus proving the causative role of the infection in the pathogenetic process of HCV-related SMZL.<sup>34</sup> SMZL associated with chronic HCV infection is often associated with mixed cryoglobulinemia and the development of cryoglobulinemic vasculitis.

## GENETIC ABERRATIONS AND MOLECULAR PATHOGENESIS

Cytogenetic abnormalities can be found in up to 80 percent of SMZL patients, most frequently consisting of complete or partial 3q trisomy (30 to 80 percent of cases) and gains of 12q (15 to 20 percent of patients). However, the most significant karyotype aberrations, considered typical of SMZL, are deletions or translocations involving 7q32, which are reported in nearly 40 percent of the cases. Other alterations involving chromosomes 8, 9p34, 12q23–24, 17p, and 18q, although not typical, are helpful for the diagnosis of SMZL.<sup>35</sup>

SMZL displays a characteristic transcriptional profile with a molecular signature consisting of overexpression of genes involved in the *AKT1*, *BCR*, and *NF-κB* signaling pathways. Mutations in signaling pathways involved in marginal zone B-cell development are also observed in nearly 60 percent of SMZL, with mutations of *NOTCH2* being the most frequent, accounting for 20 percent of the cases.<sup>36</sup> As these mutations are restricted to SMZL, they represent a potential diagnostic marker (and presumably a therapeutic target) for this lymphoma subtype.

## CLINICAL FEATURES

Isolated and generally asymptomatic splenomegaly and cytopenias are the most significant clinical characteristics of this lymphoma. The majority of patients seek medical attention because of the presence of anemia and/or thrombocytopenia, mostly related to hypersplenism rather than marrow insufficiency as a consequence of disease infiltration. Lymphocytosis is always present, and basophilic villous cells in blood may also be found. Splenomegaly is detectable upon physical examination; dyspepsia and abdominal discomfort, due to the enlarged (or sometimes markedly enlarged) spleen, are often reported. Massive



splenomegaly, frequently associated with small splenic hilar lymph nodes, is the typical feature of advanced cases.

Autoimmune phenomena can be associated with this lymphoma, a result of the production of autoantibodies sustained by the neoplastic clone. Hemolytic autoimmune anemia or immune thrombocytopenia are present in 10 to 15 percent of patients. In up to 40 percent, a serum monoclonal paraprotein can be detected.

## MORPHOLOGY

A micronodular lymphoid infiltrate of small lymphocytes typically surrounds and replaces the splenic white pulp germinal centers in SMZL, with involvement of the red pulp also consistently observed. The malignant cells resemble those of marginal zone MALT lymphoma; although some are larger and resemble centroblasts or immunoblasts. Plasmacytic differentiation is usually appreciated within germinal centers. Neoplastic lymphocytes are typically CD20+, CD23–, CD38–, CD5–, CD10–, cyclin D1–, or IgD+. Intertrabecular lymphoid nodules in the marrow mimic the morphology of tumor nodules in the spleen, with occasional reactive germinal centers surrounded by neoplastic cells. Neoplastic lymphocytes can usually be recognized in the blood,<sup>37</sup> with the classic villous morphology, characterized by the presence of polar small cytoplasmic projections (seen in only a subset of cells).

## DIFFERENTIAL DIAGNOSIS

SMZL can be distinguished from other indolent lymphomas associated with splenomegaly by the integration of clinical, morphologic, immunohistochemical, and genetic data. Both follicular lymphoma and mantle cell lymphoma may show a micronodular pattern of splenic involvement; however, the expression of both CD5 and cyclin D1 by mantle cell lymphoma and of CD10 by follicular lymphoma represents clear diagnostic clues differentiating these subtypes. A more challenging distinction is with lymphoplasmacytic lymphoma (LPL) because plasmacytic differentiation is seen in 28 percent of cases of SMZL. The presence of a very large IgM paraprotein spike (>10 g/L) favors the diagnosis of LPL rather than SMZL, though small IgM paraprotein spikes may also be seen in SMZL (<10 g/L usually).

## THERAPY

Treatment is required only in symptomatic SMZL patients with massive splenomegaly causing pain, early satiety, or cytopenias, defined as hemoglobin less than 100 g/L, platelets less than 80,000/ $\mu$ L, or neutrophils less than 1000/ $\mu$ L. Asymptomatic patients may be followed clinically without intervention for many years since treatment does not influence survival.<sup>15</sup>

At present, given the absence of randomized trials specifically involving SMZL patients, there is no consensus regarding the treatment of newly diagnosed and relapsing disease. Frontline splenectomy allows rapid correction of anemia, thrombocytopenia and neutropenia—if present—and the removal of the dominant focus of the disease, even though such management does not influence marrow infiltration or blood lymphocytosis. Postsplenectomy partial remissions are generally stably maintained for years, and patients can remain asymptomatic, with a median time to next treatment of 8 years.<sup>38</sup>

Systemic therapy is required when major contraindications to surgery exist, in elderly patients, in those who relapse or progress after splenectomy, and in case of advanced disseminated nodal disease or high-grade transformation. Rituximab, used both as a single agent and combined with chemotherapy, is highly effective in this subgroup of patients<sup>39,40</sup> and is preferred to splenectomy by some authorities. Chemotherapy regimens are based on alkylating agents (such as chlorambucil or cyclophosphamide), fludarabine, or bendamustine.<sup>41,42</sup>

## COURSE AND PROGNOSIS

The median overall survival for patients with SMZL is 5 to 10 years, although a median survival time of less than 4 years has been documented in a subset of patients presenting with advanced or aggressive disease (25 to 30 percent of cases). In 10 to 20 percent of cases, histologic transformation into a diffuse large B-cell lymphoma occurs.

A prognostic model has been developed and validated in a cohort of more than 300 patients<sup>43</sup>; the model is based on three variables: hemoglobin concentration (<120 g/L), LDH (if elevated at diagnosis), and albumin levels (<35 g/L). This model, although lacking therapeutic implications, allows stratification of patients into three risk groups, including a low-risk group (no risk factors) with an 88 percent 5-year cause-specific survival rate, an intermediate-risk group (one risk factor) with a 73 percent 5-year cause-specific survival rate, and a high risk group (at least two risk factors) with a 50 percent cause-specific survival rate.

## ● NODAL MARGINAL ZONE LYMPHOMA

### DEFINITION AND EPIDEMIOLOGY

Nodal MZL is a mature, postgerminal center B-cell lymphoma sharing many morphologic and immunohistochemical characteristics with EMZL and SMZL, although it is considered a distinct clinicopathologic subtype by the current WHO classification. It is a rare disease, accounting for less than 2 percent of all lymphomas, with a median age at onset of between 50 and 60 years. The association with autoimmune phenomena is weak for this lymphoma, in contrast to other MZLs.

### GENETIC ABERRATIONS AND MOLECULAR PATHOGENESIS

No typical cytogenetic aberrations have been demonstrated for NMZL. Among the reported abnormalities are gains of chromosomes 3, 7, 12, and 18 and structural rearrangements of chromosome 1, with breakpoints in 1q21 or 1p34.<sup>44</sup> Gain of several regions of chromosome 3 constitutes a common marker of NMZL, occurring in 20 to 25 percent of patients, and is shared with patients presenting with EMZL. More than three-quarters of patients harbor somatic *IGHV* gene mutations and show a biased use of *IGHV* segments 3 and 4. These mutations are equally seen in both HCV-positive and HCV-negative patients; however, *IGHV* gene segment 1–69 seems to be preferentially used in HCV-related cases, suggesting differential antigenic stimulation driving lymphoma B-cell precursor selection and the possible pathogenic role of HCV itself.<sup>45</sup>

### CLINICAL FEATURES

The majority of patients affected by NMZL present with disseminated peripheral and abdominal nodal involvement. Marrow infiltration is seen in less than half of the patients, and blood involvement is rare. Extranodal disease is absent, by definition, and the presence of splenomegaly should suggest a diagnosis of SMZL. Performance status is generally good, with lymphoma-related symptoms reported in 10 to 40 percent of the cases. A serum monoclonal component is detected in only 10 percent of patients.

### MORPHOLOGY

Neoplastic elements within lymph nodes have a marginal zone pattern of infiltration, with residual follicles being well preserved or expanded in some cases (“MALT-type” NMZL), diminished in other cases (“splenic-type” NMZL), or sometimes colonized by the lymphoma cells, in which case marked plasmacytoid differentiation and a nodular or follicular growth pattern mimicking follicular lymphoma is often seen (Fig. 12–2).

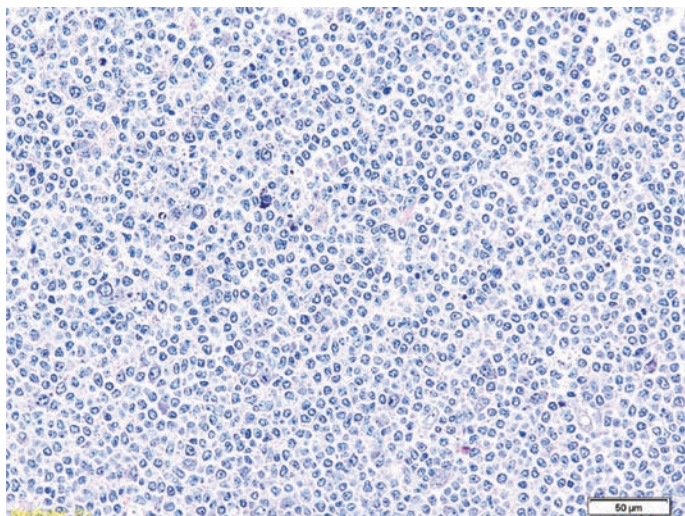


**Figure 12-2.** Nodal marginal zone lymphoma (Giemsa stain, magnification  $\times 200$ ). (Used with permission of Dr. Claudio Agostinelli.)

## DIFFERENTIAL DIAGNOSIS

Pediatric NMZL is a different clinical entity that arises in adolescent patients, with a striking predominance in males. Atypical cells within lymph nodes show a marked pleomorphism and an interfollicular distribution with marginal zones considerably expanded.<sup>1</sup> Patients generally present with localized stage I disease, which can be managed conservatively (surgical excision and observation), with low rates of disease recurrence.

The pathologic distinction between NMZL, LPL, and follicular lymphoma with marginal zone differentiation can be difficult. Immunohistochemistry may be a useful diagnostic tool by demonstrating *BCL6* and *CD10* expression in follicular lymphoma, whereas the demonstration of a large IgM paraprotein spike in the serum suggests LPL rather than NMZL.

## THERAPY

No specific treatment consensus guidelines have been developed for NMZL, and patients are generally well managed according to treatment paradigms developed for follicular lymphoma and other indolent NHLs. In limited-stage disease, surgery and radiotherapy are often appropriate, whereas immunochemotherapy is the most suitable option for patients presenting with symptomatic advanced-stage disease.<sup>15,46</sup> Rituximab

may be combined with cyclophosphamide, vincristine, and prednisone (R-CVP); fludarabine-containing and anthracycline-containing regimens (such as R-CHOP, which is based on cyclophosphamide, doxorubicin, vincristine, and prednisone); or bendamustine.<sup>46,46a</sup> A watchful waiting policy may instead be adopted in patients with low tumor burden disseminated disease.<sup>46</sup> Patients with concomitant HCV infection and no urgency for lymphoma treatment should receive antiviral treatment with interferon and ribavirin, as it results in HCV-RNA clearance and consequent tumor regression in most patients.<sup>47</sup> High-dose therapy and autologous stem cell transplantation can be considered for patients with aggressive presentations and short remission durations following standard immunochemotherapy regimens.<sup>46</sup>

## COURSE AND PROGNOSIS

The average 5-year survival of patients with NMZL is approximately 60 to 70 percent, with an estimated 5-year event-free survival of nearly 30 percent, thus indicating a high tendency of disease to relapse over time, albeit with rare involvement of extranodal sites.

## NEW AGENTS IN MARGINAL ZONE LYMPHOMA

Systemic approaches with targeted agents, used either as single agents or in combination with immunotherapy, represent a step forward to the optimization of treatment for patients for whom conventional immunochemotherapy is unsuitable and in case of relapsed or refractory disease.<sup>48</sup> The immunomodulatory drug lenalidomide,<sup>49</sup> which shows synergism with rituximab, may be used as a chemo-free approach in both untreated and relapsing patients.<sup>50,51</sup> Also, the first-generation proteasome inhibitor bortezomib is an effective chemo-free option,<sup>52,53</sup> particularly in the setting of extranodal MALT lymphoma, although it has a significant rate of peripheral nerve toxicity. Ibrutinib inhibits Bruton tyrosine kinase (BTK), a key component of the BCR intracellular signaling pathway, which is responsible for releasing and translocating NF- $\kappa$ B into the nucleus. A recent phase II study has demonstrated the efficacy and safety of ibrutinib in patients with previously treated MZL, irrespective of an extranodal, splenic, or nodal presentation; this finding confirms the biological relevance of BTK in this disease.<sup>54</sup> Also, the blockade of phosphatidylinositol 3-kinase delta, which is particularly active in B-cell malignancies, has a sound rationale as a targeted strategy in pretreated MZL patients.<sup>55</sup> An overview of targeted agents for the treatment of MZL patients is provided in Table 12-3.

**TABLE 12-3.** Targeted Agents in Marginal Zone Lymphoma

| Study                     | Drug                     | Setting                        | No. of Patients | ORR% | CR% | Median PFS* | Toxicity                                                                        |
|---------------------------|--------------------------|--------------------------------|-----------------|------|-----|-------------|---------------------------------------------------------------------------------|
| Kiesewetter <sup>49</sup> | Lenalidomide             | MALT <i>de novo</i> + relapsed | 18              | 61   | 33  | —           | Neutropenia, pruritus                                                           |
| Fowler <sup>50</sup>      | Lenalidomide + rituximab | MZL <i>de novo</i>             | 30              | 89   | 67  | 53.8        | Neutropenia, thrombocytopenia                                                   |
| Kiesewetter <sup>51</sup> | Lenalidomide + rituximab | MALT <i>de novo</i> + relapsed | 46              | 80   | 54  | —           | Neutropenia                                                                     |
| Troch <sup>52</sup>       | Bortezomib               | MALT <i>de novo</i>            | 16              | 80   | 43  | 22.0        | Neuropathy, diarrhea                                                            |
| Conconi <sup>53</sup>     | Bortezomib               | MALT relapsed                  | 32              | 48   | 31  | 25.0        | Neuropathy                                                                      |
| Noy <sup>54</sup>         | Ibrutinib                | MZL relapsed                   | 63              | 48   | 3   | 14.2        | Anemia, pneumonia, fatigue                                                      |
| Gopal <sup>55</sup>       | Idelalisib               | MZL relapsed                   | 15              | 47   | 7   | 7.0         | Neutropenia, transaminases elevation, diarrhea, colitis, pneumonia, pneumonitis |

CR, complete response rate; MALT, mucosa-associated lymphoid tissue lymphoma; MZL, marginal zone lymphoma; ORR, overall response rate; PFS, progression-free survival.

\*Median PFS is given in months.



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## CHAPTER 13

# BURKITT LYMPHOMA

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### SUMMARY

Burkitt lymphoma is one of the highly aggressive B-cell lymphomas. It was the first tumor to be etiologically associated with (1) a virus, specifically Epstein-Barr virus; (2) a specific translocation involving the *MYC* oncogene; and (3) one of the first cancers shown to be curable by chemotherapy alone. It presents in three clinically distinct forms: endemic, sporadic, and immunodeficiency associated. Burkitt lymphoma is an uncommon form of lymphoma in adults, with an incidence of approximately 1200 patients per year in the United States. Over the last decade, the definition of Burkitt lymphoma has been refined, largely as a consequence of improvements in immunohistochemical, cytogenetic, and molecular diagnostic techniques. Transcriptional profiling has more clearly defined Burkitt lymphoma at the molecular level, while whole-genome sequencing has expanded our understanding of the mutational landscape that underlies this disease. Despite these refinements in diagnostic criteria, the differential diagnosis includes several high-grade lymphomas, including a group of patients with a diagnosis defined by the World Health Organization (WHO) as intermediate between Burkitt lymphoma and diffuse large B-cell lymphoma and a new WHO provisional entity called Burkitt-like lymphoma with 11q aberration. Burkitt lymphoma is a highly curable malignancy in the modern therapeutic era. The majority of younger patients are cured with intensive chemotherapeutic regimens, and increasing efficacy has been demonstrated with reduced-intensity treatments in older patients. Remaining challenges include the optimal management of older patients, the development of therapy for patients with relapsed or refractory disease, and the translation of gains made in treatment to the management of endemic disease.

### DEFINITION AND HISTORY

Burkitt lymphoma (BL) may present in three distinct forms: endemic (African), sporadic, and immunodeficiency associated.<sup>1</sup> The endemic form (eBL) is the most common pediatric tumor in sub-Saharan Africa and

**Acronyms and Abbreviations:** B-ALL, acute B-cell lymphoblastic leukemia; BCL-U, B-cell lymphoma—unclassifiable; BET, bromodomain and extraterminal; BL, Burkitt lymphoma; B-LBL, B-cell lymphoblastic lymphoma; CODOX-M/IVAC, cyclophosphamide, doxorubicin, vincristine, methotrexate, ifosfamide, etoposide, and high-dose cytarabine, with intrathecal cytarabine and methotrexate; DHL, double-hit lymphoma; DLBCL, diffuse large B-cell lymphoma; EBER, Epstein-Barr-virus–encoded RNA; eBL, endemic Burkitt lymphoma; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; FISH, fluorescence *in situ* hybridization; GEP, gene-expression profiling; HAART, highly active antiretroviral therapy; hyper-CVAD, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone; Ig, immunoglobulin; LDH, lactate dehydrogenase; NHL, non-Hodgkin lymphoma; PTLD, posttransplantation lymphoproliferative disease; R-EPOCH, etoposide, vincristine, and doxorubicin, with bolus rituximab, cyclophosphamide, and steroids; WHO, World Health Organization.

other regions of the world where malaria is endemic. It typically presents in the jaw or maxilla and is associated with Epstein-Barr virus (EBV) infection at an early age. Although there are reports dating to as early as 1910, it was Denis Burkitt who is credited with describing this malignancy in 1958 as a common tumor in children of Uganda.<sup>2,3</sup> Originally thought by Burkitt to be a sarcoma of the jaw, it was the pathologist George O'Connor who, in 1960, concluded it was a lymphoma.<sup>4</sup> In 1964, while studying BL samples by electron microscopy, sent to them from Uganda by Burkitt, Michael Anthony Epstein, Yvonne Barr, and Bert Achong discovered EBV when they recognized viral particles were present in tumor cells,<sup>5</sup> thus helping to launch the nascent field of human tumor virology. Further studies of BL over many years led to epidemiologic associations with both EBV and malaria in Africa.<sup>6,7</sup> Tumors of a similar histologic appearance were subsequently identified in the United States, Middle East, and elsewhere (i.e., nonendemic regions) and termed *sporadic BL*. Sporadic cases were found to occur in older individuals, typically presenting in the abdomen rather than the orofacial region, and were infrequently associated with EBV.<sup>8</sup> In 1985, a third class of BL was identified in immunosuppressed patients, most commonly as a result of infection with HIV.<sup>9</sup> BL was referred to as “small noncleaved cell lymphoma” according to the Kiel classification system proposed by Karl Lennert in 1977, but this designation is no longer used. In the mid-1970s, recurrent chromosomal translocations involving chromosomes 8 and 14 were described in BL,<sup>10,11</sup> paving the way for the identification of *MYC* as an important human oncogene when it was shown to be the translocation partner involved with immunoglobulin (Ig) heavy-chain and light-chain translocations.<sup>12,13</sup> Evolution in the diagnosis and treatment of BL has occurred over the past few decades. High cure rates are now achieved among pediatric and young adult populations in health care settings capable of delivering intensive combined chemotherapy regimens, both with and without B-cell–directed monoclonal antibody therapy (i.e., rituximab). In 2006, a “molecular signature” for BL was developed from gene-expression profiling (GEP) data,<sup>14</sup> and in 2012 the first whole-genome sequences were published.<sup>15</sup> Despite these scientific and technologic advances in our understanding of this historically important disease, translation of this information into specific targeted therapeutics for BL (i.e., inhibitors of known dysregulated pathways or mutated gene products) has yet to be realized. Preclinical studies, however, indicated that proteins in the bromodomain and extraterminal (BET) family are influential regulators of gene expression, in particular *MYC*.<sup>15a</sup> Studies exploring *MYC* targeting through BET-bromodomain inhibition are ongoing.<sup>15b</sup>

### EPIDEMIOLOGY

The endemic form of BL is found in equatorial Africa (as well as Brazil, Papua New Guinea, and other malaria-endemic regions), with a peak age incidence at 4 to 7 years, and is nearly twice as frequent in boys as in girls. In Africa, it accounts for 20 percent of cancers in newborns to 14-year-olds and for the majority of non-Hodgkin lymphomas (NHLs) in all age groups.<sup>16</sup> Infection by EBV is found in nearly 100 percent of patients with eBL, and higher titers are linked to increased risk of eBL.<sup>7</sup> Although not as close, there is an epidemiologic association with malaria,<sup>17</sup> in addition to other environmental factors.<sup>16</sup> Sporadic BL, defined as cases outside endemic African regions, accounts for 1 to 2 percent of NHL, is higher in males than in females, and has a median age of 30 years. In the United States, BL exhibits primarily a bimodal age distribution, with at least two incidence peaks of approximately 10 and 75 years of age (median age of approximately 30 years), as compared to other NHLs, which generally increase from childhood through adulthood.<sup>18</sup> Immunodeficiency-related BL increased in incidence during the AIDS epidemic; however, with improved antiretroviral therapy, the incidence has decreased in the United States and countries with access to effective therapy for HIV.

## ● ETIOLOGY AND PATHOGENESIS

### MOLECULAR GENETICS

#### *Myc Overexpression and Gene-Expression Profiling*

The unifying feature of all three types of BL is activation of the *MYC* gene via an Ig translocation, leading to high levels of *MYC* protein, which activates transcription of a variety of genes involved in cell growth. Translocations are thought to occur via double-stranded DNA breaks that occur during normal class-switch reaction and somatic hypermutation in mature B-cell development, which in turn depends on activation-induced cytidine deaminase.<sup>19</sup> In addition, somatic point mutation of growth-regulatory genes (such as *MYC*), which are also caused by activation-induced cytidine deaminase, may also play an important role.<sup>20</sup> The dominant role of *MYC* activation in BL pathogenesis is emphasized by the results of multiple independent gene-expression studies. GEP analysis is capable of distinguishing BL from diffuse large B-cell lymphoma (DLBCL), and this finding is predicated on identifying cases with highly expressed target genes of *MYC*, as well as markers of germinal center B cells and lower expression of target genes of the nuclear factor (NF) kappa B pathway.<sup>21</sup> In a separate study, a core group of eight cases of pediatric BL (fulfilling World Health Organization [WHO] criteria) were used to generate a molecular signature, and tumors that matched this expression pattern were termed “molecular BL” (mBL).<sup>22</sup> Additional characteristics of this group included lower cytogenetic complexity (including near-total absence of translocations involving *BCL6* and/or *IGH-BCL2*) and the presence of *MYC* translocations involving Ig genes, rather than non-Ig partners, features that, again, distinguish BL from DLBCLs and other high-grade NHLs. In an analysis of 59 cases of mBL, 3 percent lacked *MYC* translocation but were classified as BL by gene expression classifiers. These cases shared interstitial gains and telomeric losses of chromosome 11q. These abnormalities were also found in cell lines originating from high-grade B-cell lymphomas, as well as cases of *MYC*-negative, high-grade B-cell lymphomas, closely resembling BL. This defined a unique subset of mBL characterized by recurrent dysregulation of genes in 11q.<sup>22a</sup> This finding has led to a provisional designation in the revised WHO classification called *Burkitt-like lymphoma with 11q aberration*.<sup>22b</sup>

#### *Next-Generation Sequencing and ID3/TCF3 Mutations*

Advanced sequencing analysis at the level of the whole genome, exome, and transcriptome has provided a more complete view of the spectrum of somatic mutations that occur in Burkitt lymphomagenesis and identified several recurring and previously unappreciated determinants of molecular pathogenesis. In addition to mutations in *MYC*, as many as 70 additional genes were found to be recurrently mutated in BL.<sup>23</sup> Mutations in *ID3*, *TCF3*, and *CCND3* are among the most common seen in multiple independent studies.<sup>23–25</sup> *ID3* is a member of the inhibitor of DNA binding (ID) protein family, and it is a negative regulator of transcription factor *TCF3*. The presence of biallelic inactivating mutations and/or deletions of *ID3* suggests it serves as a tumor suppressor. *TCF3*, conversely, was shown to be essential for BL cell viability, and the presence of monoallelic mutations that result in substitutions among highly conserved amino acid residues suggests gain-of-function mutations in *TCF3* may result. Overall, the mutational landscape for BL differs significantly from DLBCL. For example, relatively few *ID3* mutations were found among other B-cell lymphomas, even in those with Ig-*MYC* translocations.<sup>24</sup> *ID3* and/or *TCF3* mutations were also common among all three epidemiologic subtypes of BL. *CCND3* mutations were rare in eBL but abundant in the other two subtypes.<sup>25</sup>

## ALTERED IMMUNE STATUS AND INFECTION

### *HIV-Associated and Endemic Disease*

Underlying immune alteration likely plays a role in at least two epidemiologic subtypes of BL (endemic and immunodeficiency associated), although teasing apart the precise role of immune function in BL pathogenesis has proven challenging. Immunodeficiency-associated BL occurs predominantly in HIV-positive patients. Its occurrence does not directly correlate with CD4+ T-cell status, and BL only rarely occurs with other forms of immunosuppression. BL is different from other EBV-associated lymphoproliferative disorders that occur with advanced HIV, yet is otherwise comparable to EBV-positive posttransplantation lymphoproliferative disorders (PTLDs) that arise in severely immunosuppressed solid-organ allograft recipients.<sup>26</sup> Rather, the clinical course and pathogenesis of immunodeficiency-associated BL more closely parallels that of sporadic BL in the immunologically intact patient. In eBL, the underlying immune alteration is probably multifactorial, may be influenced by chronic malnutrition, and may stem from chronic immune activation to various stimuli, including holoendemic malaria, EBV, and possibly other environmental agents.<sup>27,28</sup>

### *Epstein-Barr Virus and Malaria*

The clear epidemiologic and immunologic link to malaria and EBV infection has led several investigators to characterize eBL as a “polymicrobial” disease.<sup>29</sup> Numerous biological mechanisms have been proposed to explain the etiologic link between eBL and each infection. For example, EBV may play a role prior to the *MYC* translocation event, simultaneously inducing proliferation (via Epstein-Barr nuclear antigen [EBNA] 2) and inhibiting apoptosis (via EBNA3A- and EBNA3C-induced epigenetic silencing of proapoptotic protein Bim [Bcl-2-interacting mediator of cell death], a key defender of *MYC*-induced tumorigenesis).<sup>30</sup> Alternatively, inhibition of apoptosis may be sustained in BL tumor cells as a result of EBNA-1 expression (the only latency-associated viral protein consistently expressed in BL), noncoding viral RNAs (including Epstein-Barr-virus-encoded RNA [EBER] and microRNAs), or possibly as a result of epigenetic reprogramming.<sup>31</sup> Precise mechanisms for the role of malaria in eBL are less clear, but it has been shown that *Plasmodium* species can stimulate B cells in a polyclonal fashion,<sup>32,33</sup> the effects of which may modulate EBV transcriptional programs within infected B cells.<sup>34</sup> Malaria infection also modulates virus-specific T-cell responses to EBV.<sup>35</sup> By simultaneously expanding EBV-infected B cells, which may dysregulate the activation-induced cytidine deaminase function and thereby increase the chance of acquiring a *MYC* translocation while perturbing the host's antiviral immune response, malaria infection may serve as a critical facilitator that brings together these otherwise-disparate pathologic events in a manner that predisposes specific populations to developing eBL.<sup>36</sup> Overall, these findings suggest that BL emerges in the setting of chronically altered but fundamentally intact immune system.

## ● CLINICAL FEATURES

The endemic (African) form often presents as a jaw or facial bone tumor. It may spread to extranodal sites, especially to the marrow and meninges. Almost all cases are EBV positive. The nonendemic or American form presents as an abdominal mass in approximately 65 percent of cases, often with ascites. Extranodal sites, such as the kidneys, gonads, breast, marrow, and CNS, may be involved. Involvement of the marrow and CNS is much more common in the nonendemic form. Patients with more than 25 percent marrow involvement with malignant cells often are referred to as having Burkitt cell leukemia (see “Blood and Marrow” section). In addition, in contrast to the endemic form, only 15 percent of the nonendemic cases are EBV positive.

Immunodeficiency-related cases often involve the lymph nodes and are associated with EBV in 30 percent of the cases. Staging using the system modified for childhood BL (Murphy staging system; [Table 13–1](#))



**TABLE 13-1. Murphy Staging System for Burkitt Lymphoma**

|                                                                            |
|----------------------------------------------------------------------------|
| Stage I: Single nodal or extranodal site excluding mediastinum or abdomen  |
| Stage II: Single extranodal tumor with regional nodal involvement          |
| Two extranodal tumors on one side of diaphragm                             |
| Primary gastrointestinal tumor with or without associated mesenteric nodes |
| Two or more nodal areas on one side of diaphragm                           |
| Stage IIR: Completely resected intraabdominal disease                      |
| Stage III: Two single extranodal tumors on opposite sides of diaphragm     |
| All primary intrathoracic tumors                                           |
| All paraspinal or epidural tumors                                          |
| All extensive primary intraabdominal disease                               |
| Two or more nodal areas on opposite sides of diaphragm                     |
| Stage IIIA: Localized, nonresectable abdominal disease                     |
| Stage IIIB: Widespread multiorgan abdominal disease                        |
| Stage IV: Initial CNS or marrow involvement (<25%)                         |

Adapted with permission from Perkins AS, Friedberg JW: Burkitt lymphoma in adults. *Hematology Am Soc Hematol Educ Program* 2008:341-348.

may be used rather than the Ann Arbor system, given that BL is largely an extranodal lymphoma.

## LABORATORY FEATURES

### BLOOD AND MARROW

Patients with bulky disease may have Burkitt cells in marrow and blood with accompanying suppression of normal blood counts. Characteristic pathologic features of BL on smear preparation are intermediate-size cells with round nuclei, multiple nucleoli, strongly basophilic cytoplasm (a consequence of the abundant polyribosomes), and the presence of lipid-filled cytoplasmic vesicles, some of which overlie the nucleus (Fig. 13-1). Rare cases, more commonly in males, may present principally with marrow and blood involvement, so-called Burkitt cell leukemia variant (previously classified as acute lymphocytic leukemia-L3, according to the former French-American-British [FAB] classification).

Serum lactic dehydrogenase (LDH) is often elevated as a reflection of the high cell turnover, especially in patients with bulky disease.

### HISTOPATHOLOGY AND CYTOLOGY

BL is characterized by monomorphic medium-size cells with round nuclei, multiple nucleoli, and basophilic cytoplasm.<sup>37</sup> Burkitt cells

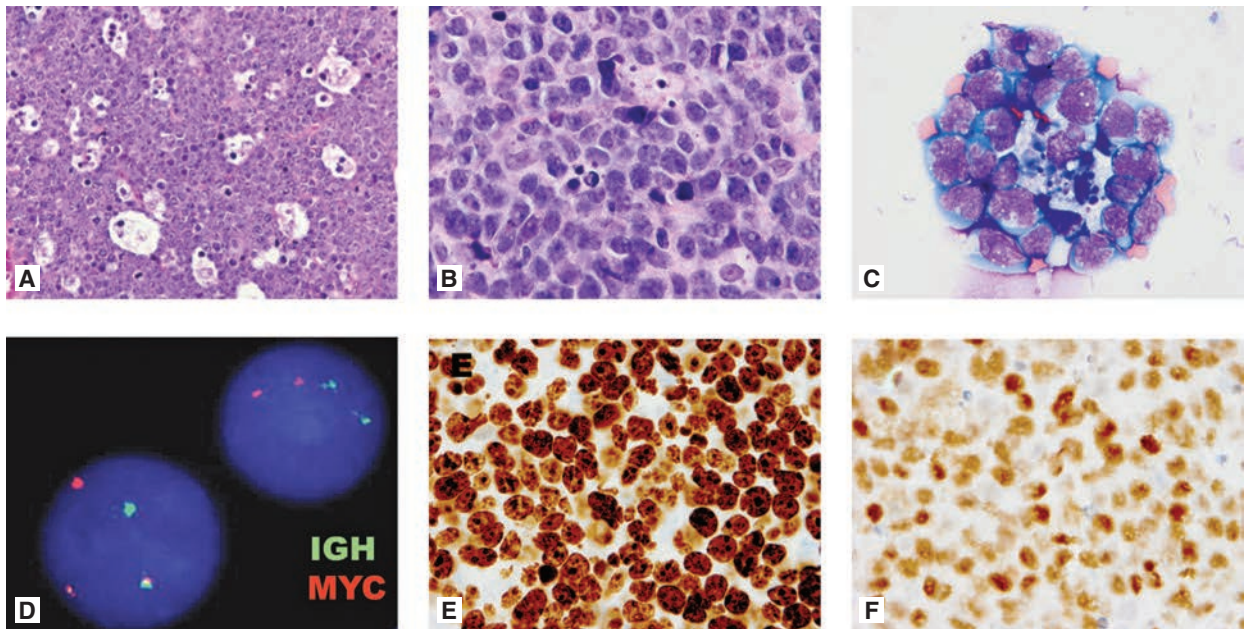


**Figure 13-1.** **A.** Lymph node biopsy section. A monomorphic population of Burkitt lymphoma (BL) cells interspersed with macrophages engorged with cellular debris as a result of the high cell turnover rate (high rate of apoptosis and cell proliferation). These “tingible body macrophages,” a term derived from a description of phagocytized nuclear debris of small lymphocytes by macrophages in germinal centers of normal lymph nodes used more than 100 years ago, are embedded in the solid monomorphic infiltrate of Burkitt cells and give rise to the “starry sky appearance,” a descriptor commonly used in lymph node and marrow sections in BL. **B.** Tumor biopsy. High-power image showing histologic detail of a monomorphic population of medium-size BL cells with rounded nuclei, relatively fine chromatin, multiple prominent nucleoli, and interspersed macrophages. **C.** Cytologic smear preparation demonstrating cluster of BL tumor cells, with characteristic vacuolated, deep-blue, basophilic cytoplasm, rounded nuclei, and multiple nucleoli. Note the associated tingible body macrophage at center. Similar cytologic features of tumor cells are seen on blood and marrow aspirate smear preparations. **D.** Fluorescence *in situ* hybridization (FISH) image demonstrating the presence of *IGH-MYC* translocation (*lower left cell*). Colocalization of the *IGH* locus probe (*labeled green*) and *MYC* probe (*labeled red*) results in a fused yellow signal consistent with t(8:14) chromosomal translocation. The *upper right-hand cell* shows a normal pattern of two red and two green nontranslocated alleles. **E.** Ki-67 immunoperoxidase stain of BL showing the very high prevalence of cells in the mitotic cycle (virtually all nuclei show the reddish-brown reaction product of the stain). The Ki-67 monoclonal antibody identifies a nuclear protein expressed throughout the cell cycle and is a marker of cell proliferative activity. **F.** MYC immunoperoxidase stain, demonstrating upregulated nuclear expression of MYC in virtually 100 percent of cells. (*A, reproduced with permission from Lichtman's Atlas of Hematology, www.accessmedicine.com. D, used with permission of Dr. A. Iqbal, University of Rochester.*)

have a very high proliferative rate ( $\geq 95$  percent as determined by Ki-67 staining), and frequent mitotic figures are usually present. BL has a diffuse pattern of growth comprising intermediate-size B cells (12- $\mu$ M diameter on a blood or marrow film) with a high nuclear-to-cytoplasmic ratio. Nuclear contours are round to oval without cleaves or folds, a key feature in the distinction from DLBCL. Nucleoli are typically multiple and small to intermediate in size, and the nuclear chromatin is relatively immature, being finely granular. Along with a high proliferation rate, these cells are characterized by a high rate of spontaneous apoptosis, leading to the characteristic “starry sky” pattern in marrow and lymph nodes—a monomorphic diffuse background of lymphoma cells that is interspersed with reactive macrophages engulfing cellular debris (so-called tingible body macrophages) (Fig. 13–1).

## IMMUNOPHENOTYPE

BL cells are mature B cells, positive for CD19, CD20, CD22, and CD79a and have monotypic surface IgM; they lack CD5 and CD23. BL cells also show immunologic similarity to germinal center cells of B-cell follicles rather than activated B cells, being positive for BCL6, CD10, Tc11, and CD38; negative for Mum-1, CD44, CD138, TdT (terminal deoxynucleotidyl transferase); and importantly little to no expression of BCL2. However, germinal center cell markers are not specific for BL since a significant proportion of DLBCL also has this germinal center cell signature.

## EPSTEIN-BARR VIRUS STUDIES

Although EBV is associated with 98 percent of eBL, it is also seen in 20 percent of sporadic cases and in 30 to 40 percent of HIV-associated cases.<sup>38</sup> It can be detected using *in situ* hybridization for EBER. Although EBV likely plays a key role in B-cell stimulation during a prelymphoma stage, the role for EBV after lymphoma development is unclear, as is whether EBV positivity is clinically meaningful. In EBV-positive endemic cases, CD21 (the EBV receptor) is expressed and is negative in most EBV-negative nonendemic BL cases. In contrast to primary effusion lymphomas and DLBCL, EBV-positive, HIV-associated BL does not express latent membrane protein 1 (LMP1) or EBNA2.

## CYTOGENETICS

Virtually all cases of BL have a translocation between the long arm of chromosome 8, the site of the *MYC* protooncogene (8q24), and one of three translocation partners: the Ig heavy-chain region on chromosome 14; the  $\kappa$  light-chain locus on chromosome 2; or the  $\lambda$  light-chain locus on chromosome 22. The translocations involving *MYC* can be detected by fluorescence *in situ* hybridization (FISH) using so-called *MYC* break-apart probes: a set of two fluorescently tagged DNA probes of two different colors that hybridize to the upstream and downstream side of the gene. In an unperturbed gene, they hybridize together within interphase cells, giving a composite color, whereas with translocation, the two fluorescently labeled probes are separated. A key feature of BL is the relative simplicity of their karyotype; in the majority of cases, the *MYC* translocation is the sole abnormality. This relatively limited degree of chromosomal change has been confirmed by microarray studies, which can detect submicroscopic chromosomal alterations.<sup>39,40</sup> Among the few changes repeatedly seen by microarray, *MYC* amplification is found in mBL cases that lack Ig-*MYC* translocation and have chromosomal 11q alterations. In general, this noncomplex cytogenetic profile for BL distinguishes it from DLBCL, which is among one of the primary considerations in the differential diagnosis with BL, as discussed next.

## DIFFERENTIAL DIAGNOSIS

### DIFFUSE LARGE B-CELL LYMPHOMA

The primary pathologic diagnosis in the differential diagnosis of BL is DLBCL.<sup>41</sup> Since the publication of the 2008 WHO guidelines,<sup>1</sup> the diagnosis of BL has been refined and diagnostic criteria have been tightened. Although the majority of BL cases, even in the adult, possess all of the criteria for the diagnosis (high mitotic rate in an appropriate morphologic and immunophenotypic setting, together with an Ig-positive *MYC* translocation), significant overlap exists with DLBCL, and a minority of cases do not fit neatly into the diagnosis of either. Typically, this arises because of the absence of key morphologic features in a lesion that otherwise resembles BL: a nonmonomorphic nuclear morphology; fewer tingible body macrophages than is typical; or an abnormal immunophenotype. Terms that were once frequently used to describe such cases, such as “atypical” BL or “Burkitt-like lymphoma,” have fallen out of favor for diagnostic purposes, and in the 2008 iteration of the WHO classification, these cases were classified as an intermediate or unclassifiable B-cell lymphoma (BCL-U; see “B-Cell Lymphoma, Unclassifiable” section). This designation acknowledged lymphomas in a subset with highly aggressive clinical behavior that were indistinguishable between BL and DLBCL. These lymphomas also had a GEP that was intermediate between both diseases, but not a specific entity.<sup>22</sup> The WHO 2016 classification eliminates BCL-U. B-cell lymphomas with rearrangements of both *MYC* and *BCL2* and/or *BCL6* are in a new category called high-grade B-cell lymphomas with *MYC* and *BCL2* and/or *BCL6* rearrangements. Consequently, the term *Burkitt lymphoma* is now reserved for a more pathologically uniform tumor type; thus, BL now arguably comprises one of the most homogeneous subtypes of aggressive B-cell NHL and exists separately from high-grade B-cell lymphomas with translocations of *MYC* and *BCL2* or *BCL6*.

### B-CELL LYMPHOMA, UNCLASSIFIABLE

To address the spectrum of overlapping clinicopathologic features exhibited by some cases that fall outside BL, the 2008 WHO classification developed a mixed classifier, termed “B-cell lymphoma, unclassifiable, (BCL-U) with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma.”<sup>42</sup> This designation was primarily reserved for cases with morphologic and/or immunophenotypic features that precluded more specific classification into either group.

### HIGH-GRADE B-CELL LYMPHOMA WITH *MYC* OR *BCL2* REARRANGEMENT

Another diagnostic term that has gained widespread use is *double-hit lymphoma* (DHL).<sup>43</sup> DHL previously referred to the subset of aggressive mature B-cell (non-Burkitt) lymphomas harboring translocations of *MYC* as well as at least one other protooncogene (most commonly *BCL2* or *BCL6*). The prognostic implication for DLBCLs that overexpress both *MYC* and *BCL2* (i.e., “double-hit” patterns of protein expression as determined by immunohistochemistry) is similar.<sup>44</sup> These lymphoma designations have been replaced by the 2016 WHO category of high-grade B-cell lymphoma with rearrangements of *MYC* and/or *BCL2*.

Rearrangements of *MYC* and *BCL2* occur in approximately 5 percent of cases. These lymphomas carry a very poor prognosis and often are treated with more aggressive chemo-immunotherapeutic approaches and, in some instances, autologous stem cell transplant. These are unique entities distinct from BL.<sup>44a</sup>



## B-CELL LYMPHOBLASTIC LYMPHOMA

Despite the fact that B-cell lymphoblastic lymphoma (B-LBL; also known as B-cell acute lymphoblastic leukemia [B-ALL]) is a malignant neoplasm of immature B-cell precursors, numerous features warrant its consideration in the differential diagnosis with BL, including anatomic distribution (marrow, soft tissues, and nodes); high proliferation index; overlapping histomorphology (medium-size round-cell morphology, finely dispersed nuclear chromatin, high nuclear-to-cytoplasmic ratio, and occasional starry sky pattern); immunophenotype (CD10 positive/CD20 variable); and propensity to occur in young patients. Distinctive features include the expression of TdT, absence of mature B-cell markers (e.g., surface immunoglobulin expression and BCL6), and the distinctive cytologic and flow cytometric features of immature blasts.

## THERAPY

### GENERAL CONSIDERATIONS

BL is a highly aggressive tumor; however, therapy with multiagent chemotherapeutic programs results in excellent long-term remission rates and long-term survival of up to 85 percent of children. Applying the same chemotherapy regimens to adults has shown dramatically improved response rates.<sup>45, 47a</sup> Risk stratification allows patients with limited disease to be treated with less-intensive therapy than more advanced cases and still achieve very high responses, although the vast majority of patients present with advanced disease. Patients with extensive disease can achieve 80 percent long-term survival. The regimens employ multiple non-cross-resistant drugs used over a short period. These drugs include high-dose cyclophosphamide, methotrexate, vincristine, prednisone, high-dose methotrexate, high-dose cytarabine, etoposide, and sometimes ifosfamide. CNS prophylaxis therapy, either intrathecal or systemic, is given in almost all patients with BL. Radiation therapy does not play a role in the treatment of BL, and use of radiation therapy for limited-stage diseases is of no additional benefit.<sup>48, 49</sup>

### TUMOR LYSIS SYNDROME

The tumor lysis syndrome is a serious metabolic complication of rapidly growing tumors, of which BL is a classic example. The syndrome is the result of the rapid destruction of tumor cells, highly sensitive to chemotherapy, and can result in hyperuricemia, hyperkalemia, hyperphosphatemia, secondary hypocalcemia, metabolic acidosis, and renal failure. In tumors such as BL, the cell death rate (and proliferative rate) may be so substantial in patients with bulky disease that the tumor

lysis syndrome may occur before therapy, so-called spontaneous tumor lysis.<sup>50</sup> Spontaneous tumor lysis is a highly morbid phenomenon with a poor prognosis for a salutary outcome from therapy and a complication with a high death rate. It occurs in patients with a high body burden of tumor, usually with abdominal disease, a common situation in BL. Its principal manifestation is the combination of hyperuricemia and azotemia. In BL a critical part of therapy is recognizing incipient or overt spontaneous tumor lysis rapidly or preventing chemotherapy-induced tumor lysis. LDH has been used as a surrogate marker for risk of tumor lysis, with a serum level twice the upper limit of normal for the laboratory in question being the threshold for urgent concern. The usual prophylactic therapy for this situation is carefully monitored hydration of at least 3 L of saline per day and either allopurinol or rasburicase to decrease serum uric acid concentration and thereby hyperuricosuria. Rasburicase acts more quickly than allopurinol and should be used if risk is considered high or if evidence of spontaneous tumor lysis is present initially.<sup>51, 52</sup> Continuous venovenous hemofiltration has also been very useful in allowing concomitant full-dose chemotherapy and preventing tumor lysis and renal failure.<sup>53, 54</sup>

### SPECIFIC REGIMENS

The specific regimens that have been developed to treat BL have historically been adapted from pediatric experience; [Table 13-2](#) depicts representative trial results, including adult patients. There are no studies directly comparing these regimens, and comparison among the single-arm studies is difficult because of lack of uniform diagnostic criteria, staging, and the heterogeneous patient populations studied. In general, shorter durations of chemotherapy (i.e., 6 months) are as good as longer (18-month) periods of treatment. Other studies have shown a dramatically improved response with use of four cycles of chemotherapy as opposed to 15 cycles. BL has a high proliferative rate, so subsequent chemotherapy cycles should be started as soon as hematologic recovery occurs. Waiting for a fixed period between cycles may lead to regrowth of resistant tumor cells between cycles.

Cyclophosphamide, doxorubicin, vincristine, methotrexate, ifosfamide, etoposide, and high-dose cytarabine, with intrathecal cytarabine and methotrexate (CODOX-M/IVAC) is among the most commonly used regimens in the United States for adults with BL, based upon an initial favorable publication from the National Cancer Institute (NCI) indicating extremely high response rates.<sup>45</sup> Two subsequent, small, phase II trials have used this regimen with minor modifications and have successfully enrolled greater numbers of older patients, demonstrating cure rates of approximately 64 percent.<sup>55, 56</sup> These cure rates are

**TABLE 13-2.** Outcome of Burkitt Lymphoma in Larger Studies

| Citation                      | Regimen                                      | No. of Patients | 2-Year Outcome                   |
|-------------------------------|----------------------------------------------|-----------------|----------------------------------|
| Magrath (1996) <sup>45</sup>  | CODOX-M/IVAC                                 | 54              | 89% (actual survival)            |
| Thomas (2006) <sup>64</sup>   | Hyper-CVAD with rituximab                    | 31              | 89% (estimated survival)         |
| Mead (2008) <sup>57</sup>     | CODOX-M/IVAC                                 | 58              | 64% (progression-free survival)  |
| Dunleavy (2013) <sup>61</sup> | Dose-adjusted R-EPOCH                        | 29              | 95% (event-free survival)        |
| Evens (2013) <sup>60</sup>    | R-CODOX-M/IVAC                               | 25              | 80% (progression-free survival)  |
| Rizzieri (2014) <sup>65</sup> | Short duration/dose intensive with rituximab | 105             | 74% (3-year event-free survival) |
| Hoelzer (2014) <sup>47a</sup> | Short duration/dose intensive with rituximab | 363             | 80% (5-year overall survival)    |

CODOX-M/IVAC, cyclophosphamide, doxorubicin, vincristine, methotrexate, ifosfamide, etoposide, and high-dose cytarabine, with intrathecal cytarabine and methotrexate; hyper-CVAD, fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone; NHL, non-Hodgkin lymphoma; R-CODOX-M/IVAC, rituximab with CODOX-M/IVAC; R-EPOCH, etoposide, vincristine and doxorubicin, with bolus rituximab, cyclophosphamide, and steroids.



substantially less than the initial report,<sup>49</sup> but still better than historical data with standard-dose regimens. A modification of this regimen in patients with aggressive lymphomas and high proliferative rate as measured by Ki-67 fraction has been proposed.<sup>57</sup> BL was strictly defined as the following: germinal center phenotype, BCL2 negative, MYC rearrangement positive, and absence of the t(14;18) or abnormalities at chromosome 3q27. Overall survival of the subgroup defined as BL was 67 percent in this group of older patients. Treatment-related mortality was 8 percent. Outcomes were similar among all age groups with the exception of patients older than age 65 years, who clearly had an inferior prognosis. These results likely reflect the true outcome of this regimen in practice. Several groups have further modified this regimen with the addition of rituximab, demonstrating superior outcomes compared with historical controls.<sup>58–60</sup> Based upon these findings and extrapolating from studies in other histologies of lymphoma, rituximab should be routinely incorporated in the treatment algorithms of BL.

The NCI has published an experience using a lower-intensity chemotherapy program consisting of infusional etoposide, vincristine, and doxorubicin, with bolus rituximab, cyclophosphamide, and steroids (dose-adjusted R-EPOCH). Nineteen patients with a median age of 25 years with generally favorable features (only 37 percent had elevated LDH, and only one patient had CNS disease) achieved a 95 percent rate of freedom from disease progression.<sup>61</sup> Intrathecal methotrexate was administered to all patients for CNS prophylaxis.

Other regimens used for the therapy of BL include fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) alternating with high-dose methotrexate and cytarabine.<sup>62</sup> The outcome of patients at a single institution (M.D. Anderson Cancer Center) with BL treated with this regimen in combination with rituximab resulted in an overall survival of 89 percent with only one death.<sup>63,64</sup> Cancer and Leukemia Group B has developed an intensive short-duration regimen consisting of cyclophosphamide, ifosfamide, methotrexate, vincristine, cytarabine, etoposide, and glucocorticoids. This regimen was combined with rituximab in a trial enrolling high-risk patients with a median age of 43 years, with 74 percent event-free survival at 3 years.<sup>65</sup>

The German Multicenter Study Group conducted a study of short-term intensive chemotherapy with anti CD-20 antibody rituximab in 363 patients aged 16 to 85 years old. Six, 5-day chemotherapy cycles consisted of high-dose methotrexate, high-dose cytarabine, cyclophosphamide, etoposide, ifosfamide, glucocorticoids, and triple intrathecal therapy. Rituximab was administered before each cycle and twice as maintenance. Patients older than 55 years were given a reduced intensity regimen.<sup>65a</sup> The rate of complete remission was 88 percent (319 of 363 patients). The 5-year overall survival was 80 percent, and the progression-free survival was 71 percent. Overall survival was correlated with patient age: adolescents 90 percent, adults 84 percent, older patients 62 percent.<sup>65a</sup>

In the only phase III randomized trial in BL, a French multicenter study evaluated 260 patients treated with chemotherapy with rituximab or chemotherapy without rituximab. With a median followup of over 3 years, rituximab-treated patients achieved better 3 year event-free survival (75 percent [95% CI 66–82]) compared to those in the group without rituximab (62 percent [53–70]).<sup>65b</sup>

No advantage of autologous stem cell transplantation in patients with BL has been observed, although incorporating planned autologous transplantation into the initial therapeutic algorithm has been used with reasonable results.<sup>66</sup>

Historically, patients with HIV have been managed with less-intensive chemotherapy because of the concern of immunosuppression-related morbidity. In the highly active antiretroviral therapy (HAART) era, HIV-positive patients with BL should be treated similarly to nonimmunocompromised patients, and the dose-adjusted R-EPOCH regimen is an excellent option for these patients. The rate of treatment failure

in HIV-positive patients with BL was significantly lower with a highly aggressive protocol when compared with patients who were treated with less-aggressive chemotherapy. In addition, patients tolerated the aggressive protocol reasonably well, particularly when HAART therapy was incorporated into the regimen.<sup>67</sup> In a study of 81 Spanish and German HIV-infected patients and BL, dose-intense chemotherapy, and rituximab, with dose adjustments for patients over 55 years of age, 80 percent of patients achieved a remission; 4-year progression-free survival was 71 percent.<sup>67a</sup>

## COURSE AND PROGNOSIS

To better define optimal therapy and outcome of adults with BL, an international effort focused on a group of adult patients with BL.<sup>68</sup> Authors of 12 large treatment series (10 prospective; two retrospective) provided outcome information for patients enrolled on their clinical trials who were older than age 40 years. In this pooled analysis, patients older than age 40 years were underrepresented in the published literature and had significantly inferior outcomes in 10 of the 12 series. Despite this, the majority of adult patients were cured using these regimens.

According to data from the NCI's Surveillance, Epidemiology, and End Results (SEER) Program, up to 30 percent of BL diagnoses in the United States include an "older" group of patients, age 60 years or older. Treatment options may be limited for this group of patients, as many older patients may not tolerate high-dose methotrexate and are not candidates for autologous transplantation. Although the number of older patients on the dose-adjusted R-EPOCH trial was limited, this regimen has demonstrated safety in elderly patients with DLBCL. In the German Multicenter Study Group trial, an adjusted chemotherapy dose was tolerated in the older group, and outcomes were encouraging (62 percent overall survival).<sup>65a</sup> A confirmatory trial conducted in several centers is ongoing and enrolling older patients.

For patients with relapsed or refractory disease, autologous transplantation is best reserved for patients inadequately treated initially, as a consolidation procedure. Patients who relapse after appropriate therapy for BL tend to have highly treatment-resistant disease, with almost uniformly unfavorable outcome. In a study of a small number of patients who had refractory disease or who relapsed after treatment with curative intent, results of additional therapy were abysmal, and the authors suggested palliative therapy should be a consideration.<sup>69</sup> They reasoned that further progress required better outcome with frontline therapy. A study of BL predictors of survival was conducted in three age groups: children age <15 years (797 patients), adults aged 40 to 70 years (1994 patients), and elderly aged >70 years (757 patients) using the SEER database of the U.S. NCI for the time interval 2000–2013. The 5-year survival rates were 90.4, 47.8, and 28.9 percent, respectively.<sup>69</sup> In children, stage IV disease and multiple primary sites were associated with worse outcomes. In adults, multiple primary sites, stage III or IV disease, being of African descent, and having primary marrow involvement were each associated with increased mortality. In the elderly, having stage II or higher disease and multiple primary sites were poor prognostic indicators<sup>70</sup>.

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## CHAPTER 14

# CUTANEOUS T-CELL LYMPHOMA (MYCOSIS FUNGOIDES AND SÉZARY SYNDROME)

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### SUMMARY

Cutaneous T-cell lymphoma (CTCL) is a heterogeneous group of malignant lymphomas that share the propensity for malignant T lymphocytes expressing cutaneous lymphocyte antigen to infiltrate the skin. Mycosis fungoides (MF) is the most common variant of CTCL, representing approximately 44 percent of all cases. Sézary syndrome (SS) is a leukemic variant of CTCL, affecting approximately 3 percent of patients.<sup>1</sup> MF and SS are malignant proliferations of mature memory T lymphocytes of the helper phenotype (CD4+CD45RO+),<sup>2</sup> which render patients immunocompromised even at the earliest stages of the disease. Advanced stages are associated with severe immune suppression. Diagnosis is established by skin biopsy, followed by staging workup, which includes radiologic imaging and pathologic evaluation of the lymph nodes, internal organs, blood, and marrow, as appropriate, according to presenting manifestations of the disease.

MF and SS are divided into early and advanced stages for therapeutic and prognostic reasons. In early stages, the disease follows an indolent course and has a favorable prognosis. In advanced stages, the prognosis is poor. There are numerous therapeutic options, but no treatment has been proven to improve survival. Nonetheless, newer studies suggest that survival of modern cohorts

is longer than historically documented cohorts.<sup>3,4</sup> Multiagent chemotherapy is inferior to immune modulating and biologic therapies.<sup>5</sup> Considering the overall protracted course of the disease, its indolent character, immunocompromised status of the patients, and absence of definitive therapy, aggressive multiagent chemotherapy contributing to immunosuppression should be reserved for end-stage palliation or as a bridge to stem cell transplantation, with the goal of definitive cure.<sup>6–8</sup> There are several FDA-approved single agents for therapy of CTCL that are safe and effective against the disease and may be used in the therapeutic ladder prior to multiagent regimens.<sup>9–11</sup> Novel single agents and combinations of new and older agents are currently in clinical trials. Because no single therapy is considered to be the standard of care for MF or SS, clinical trials remain an important option for most patients. The goal of therapy is to induce long-term remissions without compromising patients' immunity and improve the patient's quality of life.

### DEFINITION AND HISTORY

In 1806, Baron Jean-Louis Alibert described a patient who presented with skin patches that grew into plaques and mushroom-like tumors and first coined the term *mycosis fungoides* (MF).<sup>12</sup> In 1938, Sézary and Bouvain described a syndrome of pruritus, generalized exfoliative erythroderma, and abnormal hyperconvoluted lymphoid cells in the blood.<sup>13</sup> Today, this condition is referred to as *Sézary syndrome* (SS), a condition seen in a subset of patients with CTCL.

Prior to the 1970s, cutaneous lymphomas were believed to be cutaneous counterparts of the systemic lymphomas. In 1975, Lutzner and associates suggested the term *cutaneous T-cell lymphoma* (CTCL), recognizing that despite the significant similarities of malignant cell morphology and phenotype to systemic T-cell lymphomas, the cutaneous lymphomas represented distinct entities.<sup>14</sup> Although this definition has helped to distinguish cutaneous lymphomas from systemic disease, it has also led to inappropriately using the umbrella term *CTCL* interchangeably with MF. The combined World Health Organization (WHO)–European Organization for Research and Treatment of Cancer (EORTC) classification was first developed in 2005 to resolve differences among various classifications.<sup>15</sup> It has been most recently revised in 2016 and is currently accepted as the international standard for MF and SS staging and classification (Table 14–1).<sup>16,16a</sup>

### EPIDEMIOLOGY

In the United States, MF is 1.6 times as common in males as in females; the median age at diagnosis is 55 years. Americans of African descent have a higher incidence of MF and a poorer prognosis than Americans of European descent; MF occurs least often in Asians and Hispanics. Evidence for a genetic predisposition (germline transmission of susceptibility) in patients with CTCL is inconclusive. A 5.7 percent increase in incidence of CTCL per year occurred from the early 1970s to 1998, but the incidence subsequently leveled off and stabilized, with a current incidence of approximately one per 100,000 persons.<sup>17</sup> Incidence is highly age dependent, with the highest incidence of 3.6 per 100,000 in adults over the age of 70 years. Approximately 1700 new cases of MF and SS are reported annually in the United States, comprising approximately 2 percent of all lymphomas.<sup>17a</sup> Mortality rate varies widely according to the stage of disease. Stage I mortality does not differ from the mortality of age-matched controls. However, stage IV patients have a 27 percent

**Acronyms and Abbreviations:** ALCL, anaplastic large cell lymphoma; ALK-1, anaplastic lymphoma kinase-1; ATRA all-*trans* retinoic acid; BCNU, bis-chloroethylnitrosourea or carmustine; CLA, cutaneous lymphocyte antigen; CT, computed tomography; CTCL, cutaneous T-cell lymphoma; DD, denileukin diftitox; ECP, extracorporeal photopheresis; EORTC, European Organization for Research and Treatment of Cancer; FNAC, fine needle aspiration cytology; HDACi, histone deacetylase inhibitor; HTLV-1, human T-lymphotropic virus type 1; Ig, immunoglobulin; IFN, interferon; IL, interleukin; LEBT, localized electron beam therapy; LPD, lymphoproliferative disorder; LyP, lymphomatoid papulosis; MF, mycosis fungoides; MMAE, monomethyl auristatin E; NBUVB, narrowband UVB; NCCN, National Comprehensive Cancer Network; NK, natural killer; PCALCL, primary cutaneous anaplastic large cell lymphoma; PCR, polymerase chain reaction; PET, positron emission tomography; PUVA, psoralen with UVA; RXR, retinoid X receptor; SDT, skin-directed therapy; SS, Sézary syndrome; TCR, T-cell receptor; Th2, T-helper type 2; TNMB, tumor, node, metastasis, blood; TSEBT, total-skin electron beam therapy; UV, ultraviolet; UVA, ultraviolet A; UVB, ultraviolet B; WHO, World Health Organization.

**TABLE 14-1.** World Health Organization–European Organization for Research and Treatment of Cancer Classification of Primary Cutaneous T-Cell and Natural Killer Cell Lymphomas

- I. Mycosis Fungoides (MF)
- II. MF Variants and Subtypes
  - A. Folliculotropic MF
  - B. Pagetoid reticulosis
  - C. Granulomatous slack skin
- III. Sézary Syndrome
- IV. Adult T-Cell Leukemia/Lymphoma
- V. Primary Cutaneous CD30+ Lymphoproliferative Disorders
  - A. Primary cutaneous anaplastic large cell lymphoma
  - B. Lymphomatoid papulosis
- VI. Subcutaneous Panniculitis-Like T-Cell Lymphoma
- VII. Extranodal Natural Killer/T-Cell Lymphoma, Nasal Type
- VIII. Primary Cutaneous Peripheral T-Cell Lymphoma, Rare Subtypes
  - A. Primary cutaneous gamma-delta T-cell lymphoma
  - B. Primary cutaneous aggressive epidermotropic CD8+ T-cell lymphoma (provisional)
  - C. Primary cutaneous CD4+ small/medium-size pleomorphic T-cell lymphoproliferative disorder (provisional)
  - D. Primary cutaneous acral CD8+ T-cell lymphoma (provisional)
  - E. Primary cutaneous peripheral T-cell lymphoma, not otherwise specified

5-year survival, 10 percent 15-year survival, and median survival of 1.5 years.<sup>18</sup> The mortality rate of MF in the United States has been declining, possibly because of earlier diagnosis of the disease.<sup>17a,18</sup>

## ETIOLOGY AND PATHOGENESIS

The etiologies of MF and SS are unknown, although epidemiologic features are suggestive of an infectious origin, including a predilection for elderly individuals and a higher-than-expected incidence in immune-suppressed patients.<sup>19</sup> However, studies to date have failed to reveal consistent associations between any particular infectious agent and CTCL, including novel infectious agents.<sup>20</sup> A “persistent antigen stimulation” hypothesis was proposed as an initial event after MF was observed to be a disease of mature CD4+ memory T cells, but the stimulating antigen is not known.<sup>21,22</sup> CTCL patients have a high rate of colonization with *Staphylococcus aureus*. This microbe and its enterotoxins are associated with CTCL disease progression, pathogenesis, and immune dysregulation, and treatment with antibiotics can lead to clinical improvement.<sup>22a</sup> MF is also viewed as a disease of immune dysregulation. Tumor progression is associated with decreased antigen-specific T-cell responses and impaired cell-mediated cytotoxicity. On the other hand, improved survival is associated with intact cell-mediated immunity. Progression of MF is associated with progressive T-helper type 2 (Th2) skewing and increased production of Th2 cytokines. This alteration accounts for many of the immune abnormalities associated with advanced MF, such as hypereosinophilia, increased interleukin-13 (IL-13), increased serum immunoglobulin (Ig) A and IgE levels, impaired natural killer (NK) cell function, and impaired cellular immunity.<sup>22b,23,24</sup> Late-stage MF and SS are associated with declining immunocompetence,<sup>25</sup> resulting in severe

life-threatening infections and a high incidence of secondary malignancies. The latter increase is not attributable to prior treatment with carcinogenic agents alone.<sup>26</sup> Environmental factors have been suggested in the etiology of CTCL in Europe<sup>27</sup> but have not been confirmed by epidemiologic studies in the United States.<sup>28</sup>

## CLINICAL FEATURES

The clinical presentations of MF and SS are highly variable. Cutaneous manifestations of the disease result from skin infiltration by malignant cutaneous lymphocyte antigen (CLA)-positive lymphocytes and depend on the extent of skin involvement. Patients initially may present with “chronic dermatitis” that is resistant to therapy, which is often misdiagnosed as spongiotic dermatitis (so-called eczema), “psoriasis-like dermatitis,” or other chronic, nonspecific dermatoses, usually associated with pruritus. Histologically, diagnosis may be difficult, especially in the early stages of the disease and in its erythrodermic form, as the abnormal atypical infiltrate can be minimal and can be masked by normal inflammatory infiltrates in the skin, or it can be misinterpreted as a normal inflammatory infiltrate because of its mature CD4+ phenotype.

MF may progress through distinct stages of skin involvement, ranging from patch (Fig. 14-1A) to plaque (Fig. 14-1B) to tumor (Fig. 14-1C), but any type of lesion may progress or lesions may arise *de novo*. For descriptive purposes, the skin manifestations of MF are divided into patch stage (patch-only disease), plaque stage (both patches and plaques), and tumor stage (more than one tumor present, usually in the context of patches and plaques) (Fig. 14-1C). A *patch* is defined as a flat lesion with various degrees of erythema and fine scaling; it may be atrophic or poikilodermatous (Fig. 14-1D). A *plaque* is a well-demarcated, erythematous, brownish, or violaceous lesion of at least 1-mm elevation with a variable amount of scale. Tumors are dome shaped or nodular, at least 1 cm in diameter, and often without significant scaling.<sup>16</sup>

Distribution of the lesions depends on the clinical stage at presentation. In earlier stages, the lesions have a predilection for folds and non-sun-exposed body areas (“bathing trunk” distribution). In later stages, the lesions can affect the face, including development of ectropion, and other areas, such as palms and soles (keratoderma; Fig. 14-1E). Tumors may be generalized, and ulceration is common. Progression through the stages is variable but commonly occurs over several years.<sup>29</sup> Lesions usually are associated with pruritus, which may range from mild to excruciatingly severe, leading to insomnia, weight loss, depression, and suicidal ideation. Pruritus is one of the most important quality-of-life issues for these patients.<sup>30</sup>

Erythrodermic skin involvement occurs in 5 percent of patients with CTCL. Manifestations range from very faint to severe, with significant scaling, keratoderma, painful fissures of the palms and soles, nail dystrophy, and/or nail loss leading to the patient’s inability to walk and maintain daily activities. Severely inflamed or excoriated skin serves as a breeding ground for bacteria and other pathogens, with resulting localized or systemic infections.<sup>19,31</sup> Peripheral edema of the extremities may be significant in the later stages and lead to cardiovascular compromise.

Depending on the stage of presentation, patients may present with nodal and/or blood involvement and/or visceral metastases. The earliest stages of MF (e.g., stages IA and IB) may have a waxing-and-waning course, with minimal symptoms and may not have any negative prognostic implications for a patient. The more advanced stages usually, but not inevitably, present with symptoms of the disease. The symptomatology usually reflects the site and severity of involvement and ranges from being completely asymptomatic to causing severe pain, organ malfunction, or at the end stage of disease, multiorgan failure.





**Figure 14-1.** Mycosis fungoides. **A.** Erythematous atrophic patches with fine scale. **B.** Extensive patches and thicker plaques. **C.** Tumors on the background of preexisting patches and plaques. **D.** Poikiloderma. **E.** Keratoderma.

## LABORATORY FINDINGS

There is no definitive marker for MF or SS used in clinical practice, although many potential biomarkers are being studied. The diagnosis of CTCL usually is established by correlating clinical and pathologic findings. Early lesions may show polymorphic infiltration (containing mixed inflammatory cells) compatible with several benign dermatoses. Classically, MF lesions show superficial band-like (lichenoid)

lymphocytic infiltrate (Fig. 14-2A). The lymphocytes may range from small to large, with characteristic convoluted (cerebriform) nuclei. The hallmark of the malignant infiltrate in MF is epidermotropism (presence of lymphocytes in the epidermis without spongiosis) with formation of the epidermal clusters of lymphocytes around Langerhans cells termed *Pautrier microabscesses* (Fig. 14-2B). Atypical lymphocytes line up along the dermoepidermal junction and are surrounded by a halo artifact (Fig. 14-2C), which is an important feature of early disease.<sup>32</sup>

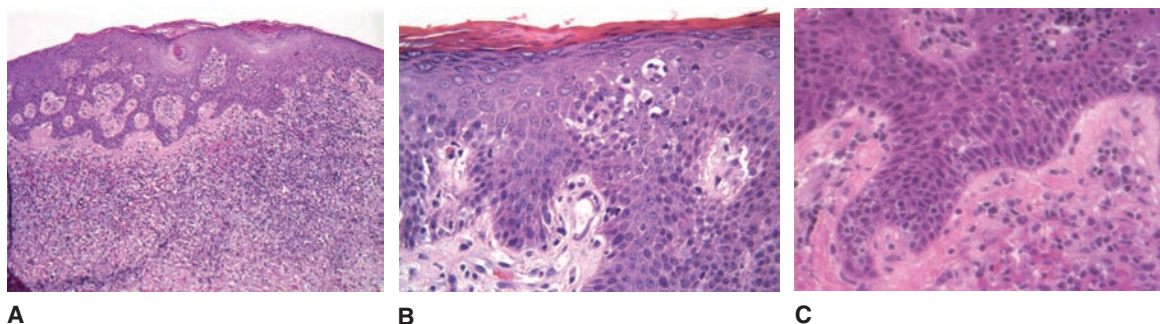


**Figure 14-2.** Mycosis fungoides. Skin biopsies stained with hematoxylin and eosin. **A.** Lichenoid (band-like) lymphocytic infiltrate in the superficial dermis. **B.** Epidermotropic atypical lymphocytes lining the dermoepidermal junction in the absence of spongiosis-forming Pautrier microabscesses. Note, halo artifact around lymphocytes at the dermoepidermal junction. **C.** Atypical lymphocytes in the epidermis.



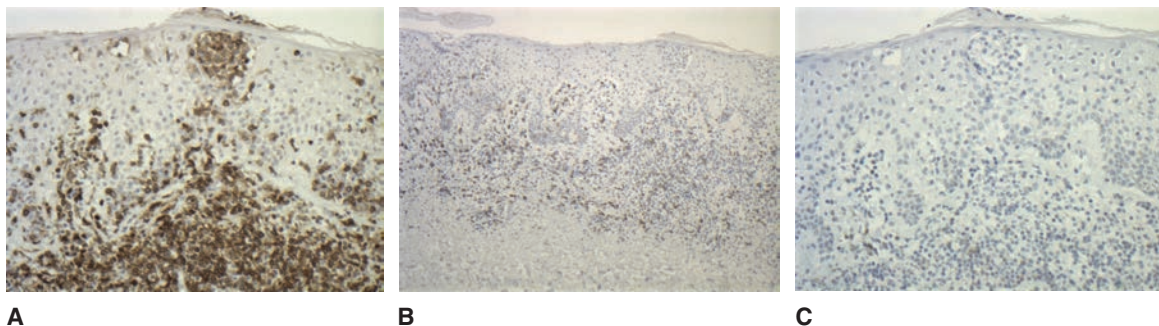


**Figure 14-3.** Mycoses fungoides. Immunohistochemistry. **A.** CD4+ lichenoid infiltrate in the superficial dermis. Note Pautrier microabscess in the epidermis. **B.** Few CD8+ cells are present. **C.** Loss of maturation marker CD7.

Superficial dermal collagen may be thickened, so-called ropey collagen. In more advanced stages, the infiltrate is less polymorphic, with a predominance of larger atypical cells extending deeper into the dermis; epidermotropism may be lost.<sup>33</sup> Transformation to large T-cell lymphoma (CD30+ or CD30-) may occur and carries a poor prognosis in the setting of MF.<sup>34</sup> Alternatively, high expression of CD30 in the setting of MF and SS may reflect concurrent presence of type C lymphomatoid papulosis (LyP) or primary cutaneous anaplastic large cell lymphoma (PCALCL) rather than transformed disease and carries a significantly better prognosis (see “CD30+ Lymphoproliferative Disorders”).<sup>34a</sup>

Immunophenotyping plays an important role in diagnosis. The cells usually are CD3+CD4+CD45RO+CD8-, a phenotype associated with mature helper-inducer T lymphocytes. Loss of maturation markers, such as CD7, and CD26 expression on CD4+ are important markers for malignant T lymphocytes.<sup>35,36</sup> The cells may express T-cell activation markers, such as HLA-DR or CD25 (IL-2 receptor). Clonal rearrangement of the T-cell receptor (TCR)  $V\beta$  gene can be identified in approximately 90 percent of advanced cases of MF, but in only 50 percent of early-stage cases.<sup>37</sup> In rare instances, the classic clinical presentation of MF may be associated with an aberrant CD4 phenotype or may have a CD4-CD8+ T-cell phenotype<sup>38,39</sup> (Fig. 14-3A to 3C). Molecular studies have identified several new markers, which may prove useful as positive markers of the disease, including thymocyte selection-associated high mobility group box factor (TOX), T-plastin (PLS3), and killer cell immunoglobulin-like receptor KIR3DL2.<sup>40-43</sup> Cytogenetic abnormalities are not consistently identified, but loss of heterozygosity on 10q and microsatellite instability may be seen in advanced-stage disease.<sup>44</sup> A possible association exists with homozygous deletion of PTEN and CDKN2A and tumor-suppressor genes on 10p and 9p chromosomes, respectively. These may be silenced with progression of disease.<sup>45,46</sup> A fluorescence *in situ* hybridization (FISH) panel has been developed to identify 11 gene copy number alterations present in 97.5 percent of patients studied with leukemic CTCL; further validation is necessary to confirm these findings.<sup>46a</sup> High-throughput sequencing (HTS) of the TCR $\beta$  CDR3 region was used to discriminate CTCL from benign inflammatory skin disorders by identifying expanded T-cell clones.<sup>46b</sup> This HTS technology is based on the specific detection of the CDR3 region on just one of the two chains of the TCR heterodimer; simultaneous definition of both chains of the TCR is currently technologically challenging. However, a method called pairSEQ is able to accurately pair TCR $\alpha$  and TCR $\beta$  sequences, linking them to lymphocytic phenotypic markers and allowing for accurate diagnosis, response monitoring, and discovery of the target epitope recognized by each TCR.<sup>46c</sup> PairSEQ could potentially revolutionize both anticancer and autoimmune therapeutics in the near future.<sup>46d</sup>

## STAGING

MF is classified according to the widely accepted modified *tumor, node, metastasis, blood* (TNMB) classification, originally adopted in 1975 by

the Mycosis Fungoides Cooperative Study Group<sup>47,48</sup> and revised to match modern developments in the field.<sup>16</sup> Accurate determination of the stage in MF and SS is of utmost importance because of its prognostic significance and critical role in selecting therapy. Cutaneous lesions are classified using the *T staging system* (Table 14-2). The area of the skin

**TABLE 14-2.** TNMB Classification of Mycosis Fungoides

### T: Skin

- T1: Limited patches, papules, or plaques covering <10% of the skin surface (T1a = patch only; T1b = plaques  $\pm$  patches)
- T2: Generalized patches, papules, or plaques covering 10% or more of the skin surface (T2a = patch only; T2b = plaques  $\pm$  patches)
- T3: At least one tumor ( $\geq 1$  cm in diameter)
- T4: Generalized erythroderma over at least 80% body surface area

### N: Lymph nodes

- N0: No clinically abnormal peripheral lymph nodes; biopsy not required
- N1: Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 1 or NCI LN0 to 2
- N2: Clinically abnormal peripheral lymph nodes; histopathology Dutch grade 2 or NCI LN3
- N3: Clinically abnormal peripheral lymph nodes; histopathology Dutch grades 3 to 4 or NCI LN4
- NX: Clinically abnormal peripheral lymph nodes; no histologic confirmation

### M: Visceral organs

- M0: No visceral organ involvement
- M1: Visceral organ involvement; requires histologic confirmation and specify organ

### B: Blood

- B0: Atypical circulating cells not present (<5%); specify “a” if flow cytometry is negative for clonal T lymphocytes or “b” if positive for clonal T lymphocytes
- B1: Atypical circulating cells present (>5%, minimal blood involvement); specify “a” if flow cytometry is negative for clonal T lymphocytes or “b” if positive for clonal T lymphocytes
- B2: Leukemia ( $\geq 1000$  cells/ $\mu$ L are Sézary cells (CD4+CD26- or CD4+CD7-), CD4/CD8 ratio  $\geq 10$ ,  $\geq 40\%$  total lymphocytes are CD4+CD7- or  $\geq 30\%$  total lymphocytes are CD4+CD26-

NCI, National Cancer Institute.

T indicates the size of the tumor and whether it has invaded nearby tissue. N indicates the regional lymph nodes that are involved. M indicates distant metastasis. B indicates whether there are tumor cells in the blood.

and type of the lesions were found to correlate with patient survival and are important prognostic predictors that need to be calculated at every visit to assess disease status.<sup>49</sup>

The extent of extracutaneous disease usually correlates with the extent of skin involvement. In early disease, significant involvement of lymph nodes and blood is unlikely. However, lymphadenopathy is present in more than half of patients as disease advances and increases with progressive cutaneous involvement. Lymph node status is designated by the *N* category in the TNMB staging of MF<sup>16</sup> (Table 14–2). Computed tomographic (CT) and positron emission tomographic (PET) scans are used to assess involvement of lymph nodes.<sup>50,51</sup> Excisional lymph node biopsy is usually recommended to assess the extent of the disease and nodal architecture and is preferred over fine needle aspiration cytology (FNAC) or core biopsy.<sup>16</sup> Although FNAC is sometimes used,<sup>52</sup> significant drawbacks include sampling error, false positives as a result of sampling dermatopathic nodes, and inability to assess nodal architecture, an important feature of nodal classification.

Histopathologic examination of affected lymph nodes may show partial or complete effacement of normal architecture, with a monomorphic infiltrate of MF cells. However, in most cases, the nodal architecture is not effaced, and dermatopathic changes are present with varying numbers of atypical lymphocytes in the T-cell paracortical areas of the node. Even the presence of dermatopathic changes alone in the lymph nodes carries prognostic significance (Tables 14–2 and 14–3).<sup>53,54</sup> Excisional biopsy of abnormal lymph nodes should be performed if the result would change management. The decision to biopsy should be weighed against the high risk of infection associated with excision in this patient population.

Metastatic disease to lymph nodes, blood, or viscera is the most significant prognostic predictor (Tables 14–2 and 14–3). Patients with visceral involvement that includes liver, spleen, pleura, and lung have a median survival of 1.4 years but comprise less than 1 percent of patients.<sup>55</sup> Blood involvement may be an important predictor of progression and survival (Figs. 14–4 and 14–5).<sup>56</sup> The number of circulating Sézary cells increases with advancing disease, and the cells are particularly prominent in patients with generalized erythroderma. However, a clonal T-cell population in the blood may be detected in up to half of stage I patients, using a highly sensitive polymerase chain reaction (PCR) technique, suggesting that early systemic disease is common.<sup>57</sup> Blood involvement is rated as the *B* category in the TNMB staging

**TABLE 14–3.** Revised Staging of Mycosis Fungoides and Sézary Syndrome<sup>16</sup>

|                  | T    | N    | M | B    |
|------------------|------|------|---|------|
| IA               | 1    | 0    | 0 | 0, 1 |
| IB               | 2    | 0    | 0 | 0, 1 |
| IIA              | 1, 2 | 1, 2 | 0 | 0, 1 |
| IIB              | 3    | 0–2  | 0 | 0, 1 |
| III              | 4    | 0–2  | 0 | 0, 1 |
| IIIA             | 4    | 0–2  | 0 | 0    |
| IIIB             | 4    | 0–2  | 0 | 1    |
| IVA <sub>1</sub> | 1–4  | 0–2  | 0 | 2    |
| IVA <sub>2</sub> | 1–4  | 3    | 0 | 0–2  |
| IVB              | 1–4  | 0–3  | 1 | 0–2  |

See Table 14–2 for definitions of T1 to T4, N0 to N3, and M0 to M1.

(Table 14–2). The B2 rating requires evidence of a T-cell clone and one or more of the following: (1) a Sézary cell count of  $\geq 1 \times 10^9$  cells/L; (2) a CD4-to-CD8 ratio of  $\geq 10$  caused by an increase in circulating T cells; and/or (3) an aberrant loss or expression of pan-T-cell markers by flow cytometry evidenced by CD4+CD7– cells comprising at least 40 percent or by CD4+CD26– cells comprising at least 30 percent of total lymphocytes. T-cell clonality can be determined by TCR gene rearrangement studies.<sup>58–62</sup> Patients with blood involvement have a higher likelihood of lymphadenopathy and visceral involvement. Marrow infiltration with extracutaneous disease occurs but is infrequently detected by biopsy despite circulating malignant cells and is not a recommended procedure for staging. The cytologic appearance of the malignant cells in visceral organs is similar to that of the malignant cells in the skin.<sup>63</sup>

In erythrodermic patients, three T4 subsets can be identified: (1) SS; (2) erythrodermic MF; and (3) erythrodermic CTCL not otherwise specified<sup>64</sup> (Table 14–4). In general, in SS, a triad of exfoliative erythroderma, generalized lymphadenopathy, and leukemia has the worst prognosis among the forms of CTCL.

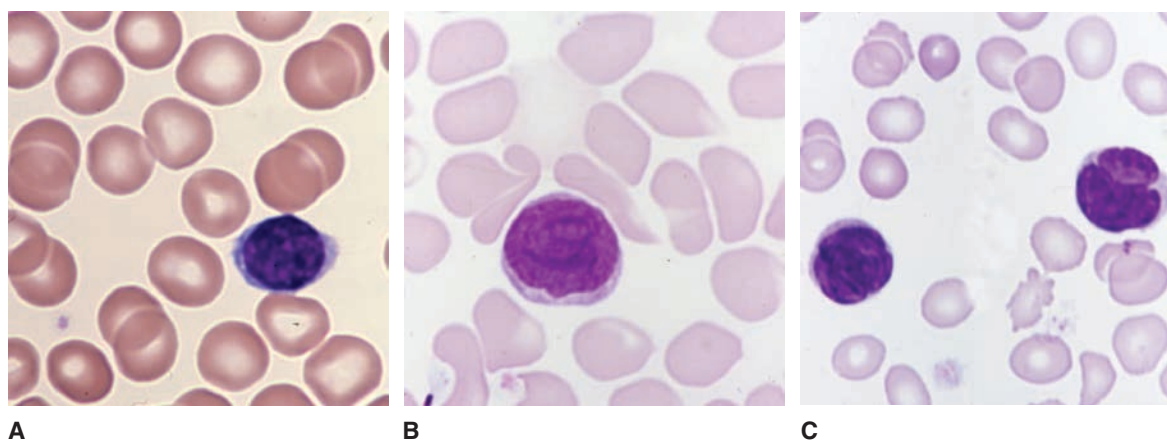


**Figure 14–4.** Blood lymphocytes. **A.** Normal small lymphocyte. **B.** Sézary cell. Note the nuclear swirls and the light microscopic appearance of the Sézary cell nucleus. Without careful inspection in cases of lymphocytosis, Sézary cells can be mistaken for small lymphocytes as seen in chronic lymphocytic leukemia. **C.** Blood lymphocytes from a patient with mycosis fungoides and disseminated disease involving marrow and blood. Note clefted appearance of the nucleus. (Reproduced with permission from Lichtman's Atlas of Hematology, [www.accessmedicine.com](http://www.accessmedicine.com).)



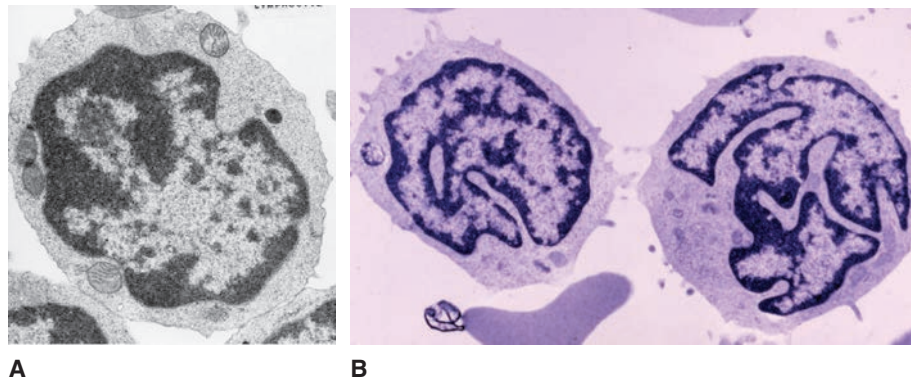


**Figure 14-5.** Transmission electron micrographs of lymphocytes. **A.** Normal lymphocyte. **B.** Two lymphocytes from a patient with Sézary cells in the blood. The latter have the striking cerebriform nuclear abnormalities characteristic of Sézary cells. (Reproduced with permission from Lichtman's Atlas of Hematology, [www.accessmedicine.com](http://www.accessmedicine.com).)

## DIFFERENTIAL DIAGNOSIS

Diagnosis of MF is based on a constellation of findings, which includes clinical presentation, skin and lymph node biopsies (if indicated), and blood evaluation. A number of benign dermatoses can mimic MF or SS and may even have clonal TCR gene rearrangements.<sup>65–68</sup> Such benign conditions include pustular or plaque psoriasis and psoriasiform dermatoses such as pityriasis rubra pilaris, seborrheic dermatitis, contact dermatitis, and eczema. Other mimics, such as parapsoriasis, intertrigo, tinea corporis, drug eruptions, alopecia mucinosa, lichen planus, atopic dermatitis, and pemphigus foliaceus, should be considered in the differential diagnosis. The diagnosis is made by appropriate investigation (e.g., potassium hydroxide skin prep), skin biopsy, and clinicopathologic correlation.

Cutaneous and systemic lymphomas other than MF should be considered in the differential diagnosis. Smoldering adult T-cell leukemia/lymphoma has a number of clinical and histologic features similar to MF, but it usually can be distinguished by the presence of antibodies to human T-lymphotropic virus type 1 (HTLV-1), and all cases have clonally integrated HTLV-1 genes in the malignant cells that can be detected by PCR. However, this distinction may be difficult.<sup>69,70</sup> CD30+ (Ki-1) lymphomas can also mimic MF, presenting as erythematous or violaceous nodules that ulcerate. The critical issue is to differentiate primary cutaneous CD30+ lymphoproliferative disorder (CD30+ LPD) from CD30+ large cell transformation of MF or secondary cutaneous involvement caused by CD30+ nodal lymphoma because of differences in prognosis<sup>34,34a</sup> (see “CD30+ Lymphoproliferative Disorders”).

**TABLE 14-4.** Classification of Erythrodermic Cutaneous T-Cell Lymphoma

| Erythrodermic Subset (T4)                                        | Preexisting MF | Blood                               |
|------------------------------------------------------------------|----------------|-------------------------------------|
| Sézary syndrome                                                  | Rarely         | Leukemia: B2                        |
| Erythrodermic MF                                                 | Always         | Normal or minimally abnormal: B0–B1 |
| Erythrodermic cutaneous T-cell lymphoma, not otherwise specified | Absent         | Normal or minimally abnormal: B0–B1 |

MF, mycosis fungoides.

**MF Variants** These rare CTCLs are classified separately from classical MF, and each type comprises less than 1 percent of all CTCLs (Table 14-1). Folliculotropic MF is characterized by a folliculotropic infiltrate that spares the epidermis, often with mucinosis, and principally affects the head and neck. It has a worse prognosis than plaque-stage classical MF.<sup>71</sup> Pagetoid reticulosis (*Woringer-Kolopp disease*) is a rare variant of MF that consists of solitary or localized cutaneous plaques, usually found on the extremities, affecting young males almost exclusively. It has a benign, indolent course, and the prognosis is excellent with no reports of extracutaneous disease or disease-related deaths.<sup>72–74</sup> It is an epidermal process, with the majority of atypical lymphocytes found within hyperplastic epidermis.<sup>75</sup> *Ketron-Goodman disease* was previously considered the disseminated form of pagetoid reticulosis, but the WHO-EORTC classification now defines the disease as aggressive epidermotropic CD8+ CTCL, cutaneous gamma/delta-positive T-cell lymphoma, or tumor-stage MF.<sup>1</sup> The histologic findings are similar to those found in Woringer-Kolopp disease, with predominantly epidermal involvement of malignant cells and a poor prognosis.<sup>76</sup> Granulomatous slack skin is another extremely rare but indolent variant of MF that causes slowly progressive laxity of skin folds and granulomatous clonal T-cell infiltrate on histology.<sup>77</sup>

## THERAPY

A variety of therapeutic modalities produce remissions in most patients with CTCL. In general, CTCL therapy is divided into (1) skin-directed therapy (SDT) and (2) systemic therapy (Table 14-5). SDT is the primary form of therapy in early disease but also is used as an adjunct in systemic disease. Therapeutic decisions may be difficult and heavily depend on the stage at presentation. Revised practice guidelines are available on the National Comprehensive Cancer Network (NCCN) website (<https://www.nccn.org>). Figure 14-6 provides an overview of the treatment algorithm for MF/SS. In view of the protracted course of the disease and lack of curative therapies, therapeutic decisions should generally be focused on induction of long-term remissions with the least toxic agents and regimens. A number of highly effective monotherapies were recently shown to be safe and effective in prospective clinical trials in patients with MF and SS, leading to their approval by the FDA in the United States and abroad. Several novel agents are in clinical trials to test for their efficacy as monotherapy for MF. In addition, there are number of promising combination regimens being tested in clinical trials for advanced MF or SS refractory to monotherapy.



**TABLE 14-5.** Therapeutic Options for Mycosis Fungoides and Sézary Syndrome

| Skin-Directed Therapy                  | Systemic Therapy                                                                                                                    |
|----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| <b>Topical Therapy</b>                 | <b>Retinoids</b>                                                                                                                    |
| Topical glucocorticoids                | Oral bexarotene                                                                                                                     |
| Topical calcineurin inhibitors         | Acitretin                                                                                                                           |
| Tacrolimus                             | Isotretinoin                                                                                                                        |
| Pimecrolimus                           | <b>Histone Deacetylase Inhibitors</b>                                                                                               |
| Topical chemotherapy                   | Vorinostat                                                                                                                          |
| Nitrogen mustard (mechlorethamine HCl) | Romidespin                                                                                                                          |
| Carmustine (BCNU)                      | <b>Immunomodulators</b>                                                                                                             |
| Topical retinoids: bexarotene          | Interferon alfa, interferon gamma                                                                                                   |
| Topical immunomodulators: imiquimod    | Extracorporeal photopheresis                                                                                                        |
| <b>Light Therapy</b>                   | <b>Monoclonal Antibodies and Conjugates</b>                                                                                         |
| NBUVB and PUVA                         | Alemtuzumab                                                                                                                         |
| Photodynamic therapy                   | Brentuximab vedotin                                                                                                                 |
| <b>Radiation Therapy</b>               | <b>Proteasome Inhibitors</b>                                                                                                        |
| Electron beam: localized, total skin   | Bortezomib                                                                                                                          |
|                                        | <b>Chemotherapy</b>                                                                                                                 |
|                                        | Oral methotrexate                                                                                                                   |
|                                        | Pralatrexate                                                                                                                        |
|                                        | Other: mechlorethamine, cyclophosphamide, chlorambucil, methotrexate, bleomycin, doxorubicin, fludarabine, pentostatin, gemcitabine |
|                                        | <b>Allogeneic Stem Cell Transplant</b>                                                                                              |

BCNU, *bis*-chloroethylnitrosourea; NBUVB, narrow-band ultraviolet light B; PUVA, psoralen plus ultraviolet light A.

## SKIN-DIRECTED THERAPIES

### Topical Glucocorticoids

Topical glucocorticoids are effective during early stages of MF. They are limited to temporary short-term use because of suppression of collagen synthesis (skin atrophy), striae formation, skin fragility, and secondary infections. The class of topical preparation depends on the area and the site of involvement. Ultrapotent topical glucocorticoids should not be used on the face, neck, and intertriginous areas. Topical steroids are rarely used as monotherapy but may be effective as an additional modality for symptomatic relief of pruritus.

**Topical Calcineurin Inhibitors** Topical tacrolimus and pimecrolimus are approved for use in atopic dermatitis. They are used off label for CTCL and are as effective as mid- to low-potency glucocorticoids for use on facial skin and intertriginous areas in patients with MF. A major advantage of these agents over steroids is that they do not suppress collagen synthesis to cause skin atrophy.<sup>79</sup> However, because the use of calcineurin inhibitors in CTCL is controversial due to theoretical risk of lymphoma,<sup>81</sup> their topical use should be limited to short-term use on small areas. Pimecrolimus is currently in clinical trials as targeted therapy for patients with PLCG1 mutations.<sup>80</sup>

### Topical Nitrogen Mustard (Mechlorethamine Hydrochloride)

Topical nitrogen mustard is a topical alkylating agent approved by the FDA for stage IA and IB patients after one prior treatment. It can also be used as a part of combination therapy with systemic agents in advanced stages. Prior to the 2013 FDA approval of mechlorethamine hydrochloride 0.016 percent gel,<sup>82</sup> it was only available through certified compounding pharmacies in forms of solution, cream, or ointment of various concentrations (0.01 to 0.04 percent). Response rates up to 80 percent in stage IA disease and up to 68 percent in stage IB can be achieved.<sup>83</sup> The major advantage of this topical therapy is its relatively low toxicity. Disadvantages include the inconvenience of daily application to large areas of skin, allergic reactions in up to half of cases,<sup>84</sup> the potential for development of skin cancer,<sup>85</sup> and the inability to cure the disease. It is approved for use from the neck down and applied three times weekly up to daily as tolerated. Therapy is usually continued for up to 12 months in responders. Frequency then is reduced to every other day for an additional 1 to 2 years. Therapy is discontinued after 3 years or when cutaneous lesions disappear completely. Mechlorethamine should not be used together with ultraviolet A (UVA) or ultraviolet B (UVB) therapy because of cumulative carcinogenic effects on the skin and significant increase in risk for skin cancer and melanoma. It is also contraindicated in pregnancy.

**Topical Carmustine (*Bis*-Chloroethylnitrosourea [BCNU])** BCNU is not currently widely used for treatment of MF because of its severe irritant reactions and its absorption from the skin, which results in systemic toxicity. The preparation ranges from 20 to 40 mg/dL in petrolatum ointment. It is applied at night and washed off in the morning. Monitoring includes biweekly complete blood counts to identify marrow suppression. Carmustine causes irreversible skin thinning, telangiectasias, and hyperpigmentation.<sup>86</sup>

**Topical Retinoids** Bexarotene 1 percent gel is a topical retinoid (retinoid) FDA approved for treatment of MF patients who are refractory to at least one other topical therapy. It is a small lipophilic molecule related to vitamin A. It readily crosses the cytoplasmic membrane and binds to nuclear receptors (retinoid X receptors [RXRs]), resulting in changes in gene expression mediated through specific intracellular receptors, leading to maturing and apoptosis of malignant cells.<sup>87</sup> A phase III clinical trial reported 44–54 percent overall response rates in stage IA–IIA disease with complete responses of 2–10 percent.<sup>88</sup> It is applied twice daily in a thin layer to the patches and plaques. The major toxicity is irritation at the site. Oral administration of bexarotene is associated with severe birth defects. Considering potential absorption of the drug from the skin surface, topical bexarotene should not be given to pregnant women.

**Phototherapy** Phototherapy is a well-established, effective treatment for MF, utilizing radiation of the UVA and UVB spectra. It is not FDA approved for treatment of MF and SS because of a lack of prospective clinical trials but is considered to be one of the most effective therapies for early disease (mainly patches and thin plaques). Phototherapy may result in complete clearing of the lesions. It was hypothesized that the mechanism of action of this therapy is depletion of Langerhans cells from the epidermis.<sup>89</sup>

The peak of therapeutic effectiveness of UVB is within 295 to 313 nm. Conventional broadband UVB lamps emit wavelengths ranging from 280 to 330 nm, but narrowband UVB (NBUVB) emits only wavelengths from 311 to 312 nm, eliminating harmful UV rays below 300 nm, which can cause erythema or severe burning and increase the risk of skin cancer.<sup>90–92</sup> Similarly, excimer lasers emitting at 308 nm can be successfully used for hard-to-reach areas resistant to other therapy.<sup>93</sup> Therapy should be instituted three times per week. On average, 6 to 12 weeks are required to achieve response. Maintenance therapy is required for at least 2 more months after a response occurs, but thereafter the maintenance regimen

## Therapies for Cutaneous T-Cell Lymphoma

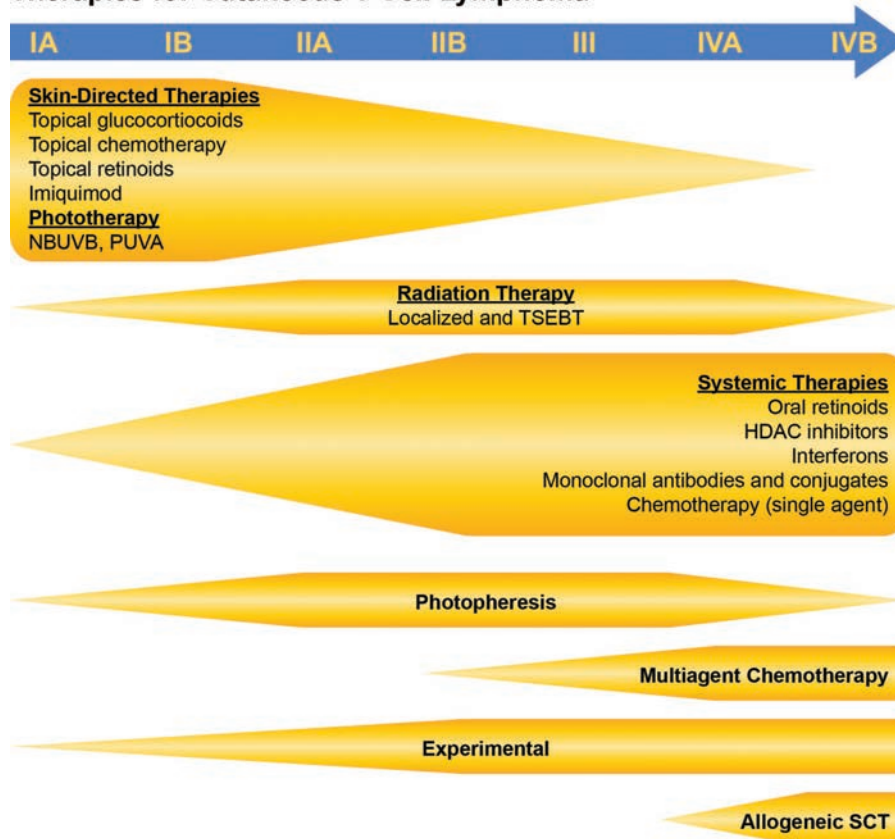


**Figure 14-6.** Cutaneous T-cell lymphoma treatment algorithm. HDAC, histone deacetylase; NBUVB, narrowband ultraviolet B; PUVA, psoralen plus ultraviolet A; SCT, stem cell transplant; TSEBT, total-skin electron beam therapy.

for various light sources is not well established and depends on the personal experience of the treating physician. NBUVB is most appropriate for patch-stage disease.

The UVA spectrum ranges from 320 to 400 nm, which penetrates deeper than UVB into the dermis, allowing treatment of thicker plaques. Phototherapy involving UVA radiation is used with psoralen and is referred to as PUVA. Psoralen is a phototoxic furocoumarin activated by UVA light. In its active form, psoralen bonds covalently and irreversibly to DNA. Therefore, psoralen activated by UVA light affects cells primarily in the epidermis and papillary dermis. A 60 percent complete remission rate and long-term remissions (>10 years) have been reported with PUVA; patients with generalized erythroderma and tumors have lower response rates than patients with plaques.<sup>94-96</sup> Psoralen usually is given at a dose of 0.6 mg/kg orally, 2 hours before the UVA light therapy. Treatments initially are given three times per week. Maintenance therapy may be given every 2 to 4 weeks for an indefinite period. Adverse effects of PUVA therapy include mild nausea, pruritus, and sunburn-like changes, with atrophy and dry skin. PUVA is not cross-resistant with other treatment modalities. Long-term side effects include an increased incidence of skin cancers and melanoma.<sup>97</sup> NBUVB may be a viable alternative to PUVA, with similar response rates in a small cohort.<sup>98</sup>

Disadvantages of phototherapy include the necessity to visit the physician's office frequently (from three times a week to once a month) and its expense. Both NBUVB and PUVA should be avoided in patients with a prior history of skin cancers or chronic actinic keratoses and contraindicated in photosensitive dermatoses.<sup>99</sup>

**Photodynamic Therapy** Photodynamic therapy is a photochemotherapy that utilizes two properties of porphyrins: their selective

accumulation in tumor sites (e.g., 5-aminolevulinic acid) and their ability to generate cytotoxic oxygen species at the tumor site after red-light irradiation. 5-Aminolevulinic acid is a natural porphyrin precursor and upon irradiation is converted in the tumor to the highly photoactive endogenous protoporphyrin IX. Red-light irradiation is safe and penetrates deeply in the tissue, allowing for treatment of thick tumors. Photodynamic therapy is especially useful in patients with localized plaques or tumors; however, the pain induced during irradiation limits its use for larger areas.<sup>100,101</sup> It is used for therapy of MF off label.

**Electron Beam Therapy** Radiation in the form of electron beam therapy is one of the oldest and the most effective forms of treatment of MF, appropriate for patients who have failed the aforementioned skin-directed therapies. It can be used as a localized therapy (LEBT) to specific sites or lesions, such as tumors, or as radiation of the entire skin surface (total skin electron beam therapy [TSEBT]). It delivers a uniform dose from the surface to a specific depth, after which the dose falls off rapidly, penetrating the dermis but sparing deeper normal tissues. Response rates range from 40 to 98 percent, depending on disease involvement, but about half of stage IA patients will relapse within 10 years.<sup>102,103</sup> Another study showed that 80% of complete responders relapsed at a median time of 4 months.<sup>104</sup> The conventional dose for TSEBT is 30–36 Gy over 8–10 weeks. Although this dose achieves a higher likelihood of complete response compared to lower doses, it carries significant side effects, such as alopecia, skin atrophy, destruction of skin adnexa, dermatitis, and increased risk of cutaneous malignancy.<sup>105-108</sup> Toxicity prevents repeated courses of treatment. Low-dose TSEBT (12 Gy over 3 weeks) has been shown to be nearly as effective, with a notable reduction and transience of adverse effects and overall response rates of

88 percent in stage IB to IIIA disease, still superior to other skin-directed therapies for MF other than conventional-dose TSEBT. Low-dose TSEBT provides a rapid time to response (median 7.6 weeks) and can be repeated multiple times.<sup>78</sup> Following radiation, patients should be transitioned to another skin-directed or systemic therapy to maintain their response.

**Imiquimod** Imiquimod 5 percent cream is a topical immunomodulator that is extremely effective in the treatment of condylomata acuminata, actinic keratoses, basal cell carcinomas, keratoacanthomas, and other cutaneous malignancies. It is a toll-like receptor-7 and -8 agonist that induces pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  and interferons, resulting in activation of a Th1-type immune response and rejection of cancer or virally infected cells.<sup>108a</sup> Several groups reported the effectiveness of imiquimod in early patch MF.<sup>109,110</sup> It should be used three times per week for 3 months. It is not FDA approved for therapy of MF and SS.

## SYSTEMIC THERAPY

### Oral Retinoids

**Oral bexarotene** is an FDA-approved, RXR-selective retinoid, or “rexinoid,” for therapy of MF. The topical formulation is discussed in “Topical Retinoids.” It is a first-line systemic agent for patients without contraindications to retinoids. Its use is recommended beginning with refractory or persistent stage IA disease through more advanced stages (NCCN guidelines). At the currently FDA-approved dose of 300 mg/m<sup>2</sup> per day, the overall response rate to bexarotene monotherapy ranges from 45 to 57 percent, with at least 2 percent complete responses.<sup>111,112</sup> Higher doses are associated not only with higher response rates and shorter time to response, but also with a higher incidence of adverse events. All patients on bexarotene rapidly develop central hypothyroidism and hyperlipidemia (most significantly hypertriglyceridemia), requiring co-administration of thyroid supplements, lipid-lowering agents, and a high-protein, low-fat, low-carbohydrate diet. Other rare adverse events include headaches, possibly a result of pseudotumor cerebri; leukopenia; hepatotoxicity; and pruritus. Standard procedures for management of patients on bexarotene therapy have been reviewed.<sup>113</sup> The majority of side effects are indicated by laboratory findings and are dose dependent; bexarotene is usually well tolerated by patients. Bexarotene is safe to use long term for maintenance therapy. Bexarotene and other retinoids are labeled pregnancy category X and must not be given to a pregnant woman or a woman who intends to become pregnant.

Other retinoids have been used for treatment of MF and SS, including isotretinoin, acitretin, etretinate (not available in United States), and all-*trans* retinoic acid (ATRA). Activity of these compounds in MF/SS has been demonstrated in case series or small open-label pilot studies, but there are no prospective studies formally evaluating these drugs.<sup>114</sup>

**Histone Deacetylase Inhibitors (HDACis)** These newer agents are epigenetic modifiers that alter gene expression and cause a variety of effects against malignant T cells. Two HDACis, vorinostat and romidepsin, are FDA approved for relapsed or refractory CTCL.

Vorinostat (suberoylanilide hydroxamic acid) is an oral pan-HDACi FDA approved for treatment of cutaneous manifestations of recurrent, refractory, or persistent MF and SS in 2008 at the dose of 400 mg by mouth daily.<sup>115</sup> Vorinostat was evaluated in an open-label, phase IIB clinical trial and was shown to have an overall response rate of about 30 percent in patients with stage IIB or greater. One patient of 74 achieved a complete response. The most common drug-related adverse events were diarrhea, fatigue, nausea, and anorexia; most were grade 2 or lower. Grade 3 or higher adverse events included fatigue, pulmonary embolism, thrombocytopenia, and nausea. Loss of taste has also been reported.

Romidepsin (desipeptide) is a selective HDACi given as an intravenous infusion. Romidepsin was FDA approved in 2009 for CTCL patients who had received one prior therapy based on a phase IIB clinical trial in patients with refractory CTCL documenting an overall response rate of 34 percent, with 6 percent complete responses.<sup>116</sup> The treated patients had a median of four prior treatments. The median duration of response was 13.7 months. Toxicities included nausea, fatigue, vomiting, and transient thrombocytopenia and granulocytopenia. Due to a low risk of cardiac arrhythmias and QTc prolongation, baseline and routine electrocardiograms should be performed, and preinfusion potassium and magnesium supplementation is necessary. Romidepsin was shown to have single-agent clinical activity with significant and durable responses in patients with CTCL.<sup>116</sup> It is given as a weekly 4-hour infusion of 14 mg/m<sup>2</sup> on days 1, 8, and 15 of a 28-day cycle.<sup>116a</sup>

Overall, HDACis have notably lower rates of grade 3 and 4 neutropenia, febrile neutropenia, or sepsis compared to systemic chemotherapy, reducing potential hospitalizations due to infection and need for aggressive infection prophylaxis.<sup>116b</sup> Panobinostat and belinostat are other HDACis approved for multiple myeloma and peripheral T-cell lymphoma, respectively, but studies showed less than 20 percent overall response rates in CTCL.<sup>116c,116d</sup>

HDACis may be excellent combinational agents, potentiating effects of other therapies, possessing radio- and photosensitizing effects. Clinical trials combining HDACis with other systemic agents are under way, and preclinical studies have shown the potential for combinations with skin-directed therapies like nitrogen mustard and electron beam.<sup>106,116e</sup>

**Interferons** Interferon (IFN) alpha and interferon gamma are immunomodulatory polypeptides that both stimulate a Th1 immune response, among their many functions. IFN alfa-2a and IFN alfa-2b are available as nonpegylated recombinant forms and pegylated forms, the latter of which are less commonly used due to lack of studies in CTCL. The response rate for IFN alfa monotherapy is 50 to 70 percent at doses beginning at 3 to 5  $\times 10^6$  units/day or three times per week, either subcutaneously or intralesionally.<sup>117</sup> Frequency and dosing depend on the type of IFN alpha and provider preference. Adverse effects include major depressive disorder, acute flu-like symptoms and fatigue, and bone marrow suppression. Compliance with frequent injections may be difficult. It can be administered as monotherapy and has been used in combination with extracorporeal photopheresis, PUVA, retinoids, methotrexate, or TSEBT. Although clinicians have reported favorable results with combination therapy, reviews of the evidence have not supported definitive superiority over monotherapy, and randomized clinical trial data are limited.<sup>10,118,119,119a</sup> A systematic review concluded that combining IFN alfa with PUVA or a retinoid may be beneficial to early-stage nonresponders of monotherapy, and combining with methotrexate may benefit advanced-stage patients.<sup>119b</sup>

Recombinant IFN gamma-1b can be administered subcutaneously thrice weekly to daily to treat CTCL. The recommended dose is 50  $\mu$ g/m<sup>2</sup> in patients with greater than 0.5 m<sup>2</sup> body surface area, and 1.5  $\mu$ g/kg in patients with less than 0.5 m<sup>2</sup> body surface area. Although studies supporting IFN gamma in CTCL are limited, some CTCL centers use it extensively as part of multimodal treatment, and it has been reportedly used in combination with bexarotene, extracorporeal photopheresis (ECP), TSEBT, vorinostat, and NB-UVB with clinical responses.<sup>119b</sup> IFN gamma may be efficacious for CTCL refractory to IFN alfa.<sup>119c</sup> The most common side effects are similar to IFN alfa with fever and flu-like symptoms and may also include nausea, headache, weight loss, dose-dependent cytopenias, liver function enzyme abnormalities, nonscarring alopecia, and autoimmune phenomena.<sup>119b,119c</sup>

**Extracorporeal Photopheresis** PUVA can be delivered by an extracorporeal technique.<sup>120,121</sup> White blood cells are collected by



leukapheresis, exposed to a photoactivating drug, and irradiated with UVA. The cells then are reinfused into the patient. The effect may be both a direct cytotoxic effect on the tumor cells and an immunologic effect by activating normal lymphocytes against the tumor cells. ECP was FDA approved in 1998 for the palliative treatment of CTCL. ECP typically is administered every 2 to 4 weeks until clearance of disease. Side effects are minimal and may be related to fluid shifts during the procedure.<sup>97</sup> A recent retrospective review suggested a survival advantage for patients treated with ECP<sup>122</sup> and beneficial responses in patients with early-stage MF.<sup>123</sup>

**Monoclonal Antibodies** Alemtuzumab is a humanized IgG1 monoclonal antibody that targets the CD52 antigen and is FDA approved for B-cell chronic lymphocytic leukemia and used off label for CTCL as salvage therapy. A response rate of 50 percent has been reported in a small cohort of CTCL patients.<sup>124,125</sup> Alemtuzumab effectively depletes leukemic cells from blood of SS patients. Low doses were shown to be safe and effective in very elderly SS patients,<sup>126</sup> and very low doses on an as-needed basis were effective long term for SS in another study.<sup>127</sup> Though alemtuzumab is capable of inducing long-term remission in SS patients, it is not effective in MF and CTCL with large cell transformation. It has a high rate of adverse events, including profound lymphopenia in most patients and grade 3 or higher infections in 62 percent of patients and should be utilized judiciously.<sup>127a</sup>

Other monoclonal antibody therapies are under study. The anti-PD-1 antibodies pembrolizumab and nivolumab, highly successful in the treatment of metastatic melanoma, have shown some promise in CTCL in preliminary studies.<sup>127b</sup> Mogamulizumab is a humanized IgG1 monoclonal antibody that targets CCR4 on CTCL tumor cells. It has shown clinical efficacy in phase I/II trials and quantitatively reduces CCR4+ circulating malignant cells.<sup>127c</sup> The KIR3DL2 receptor has been identified as both a diagnostic and a therapeutic target in CTCL. IPH4102, an anti-KIR3DL2 humanized cytotoxicity-inducing antibody, is currently in phase I trials.<sup>127d</sup>

**Monoclonal Antibody Conjugates** Brentuximab vedotin is an anti-CD30 antibody conjugated via a protease-cleavable linker to the potent antimicrotubule agent monomethyl auristatin E (MMAE). Following binding to CD30, brentuximab vedotin is rapidly internalized and transported to lysosomes, where MMAE is released and binds to tubulin, leading to cell cycle arrest and apoptosis. Brentuximab was recently FDA approved for CD30+ CTCLs, showing durable antitumor activity with a manageable safety profile in patients with relapsed/refractory primary cutaneous CD30+ LPDs.<sup>128</sup> The overall response rate was 73 percent, with 54 percent for MF and 100 percent for all other tumor types (LyP and PCALCL). Response was not correlated with level of CD30 expression at baseline in MF. Peripheral sensory neuropathy is a significant adverse event; symptoms are cumulative and dose related. Multiple ongoing trials are currently evaluating brentuximab vedotin in combination with biologics, immunotherapies, and radiation in both newly diagnosed and relapsed/refractory patients.

**Recombinant Fusion Proteins** Denileukin diftitox (DD) is an IL-2 diphtheria toxin fusion protein targeted to malignant cells that express the CD25 component of the IL-2 receptor; DD had full FDA approval in October 2008 following a phase III randomized, double-blind, placebo-controlled trial.<sup>129</sup> Side effects were numerous, including a capillary leak syndrome, infection, hepatitis, increased fluid retention, rash, shortness of breath, and flu-like symptoms such as chills, fever, weakness, bone and muscle pain, headache, nausea, and vomiting. Cardiac arrhythmias and thrombotic emergencies have been reported occasionally. Usually, adverse events are most severe during the first two cycles, diminishing as the treatments continue. DD manufacturing was discontinued as of June 2012, and a new formulation of the same product, E7777, is in clinical trials at this time.

**Proteasome Inhibitors** Bortezomib inhibits activity of the 20S unit of the proteasome to promote apoptosis in tumor cells and is approved for multiple myeloma and mantle cell lymphoma but not approved for CTCL. A small phase II trial that included 10 MF patients demonstrated a partial response in six patients and complete response in one patient; the last response lasted over 12 months. Dose-limiting side effects included myelosuppression and sensory neuropathy. Bortezomib is currently being evaluated as a combinational agent with various compounds in CTCL.

**Chemotherapy** Several agents have been FDA approved for therapy of various subsets of MF and SS.

Pralatrexate is a novel antifolate with high affinity for reduced folate carrier-1, FDA approved for peripheral T-cell lymphoma at 30 mg/m<sup>2</sup> per week. The recommended regimen for MF was identified as 15 mg/m<sup>2</sup> a week for 3 of 4 weeks; the response rate was 45 percent.<sup>130</sup> The most common grade 3 adverse event was mucositis (17 percent); the only grade 4 adverse event was leukopenia (3 percent). MF patients were not able to tolerate the "lymphoma" dosage of pralatrexate (30 mg/m<sup>2</sup> a week for 6 of 7 weeks) because of intolerable toxicities, including severe mucositis. It was hypothesized that the higher incidence of mucositis was a result of distribution of malignant cells from the skin and mucosal surfaces beyond visible lesions, resulting in "localized" tumor necrosis in the skin with collateral damage to surrounding tissues. Administration of a single 10- to 15-mg dose of leucovorin 24 hours following pralatrexate infusion completely abrogated these side effects and allowed therapy with 30 mg/m<sup>2</sup> without sacrificing efficacy.<sup>131</sup>

Systemic alkylating agents used to treat MF and SS include intravenous nitrogen mustard, cyclophosphamide, or chlorambucil. Response rates of 60 percent, with 15 percent complete remissions, have been reported.<sup>132,133</sup> Similar results are obtained with methotrexate 2.5 to 10 mg/day orally<sup>134</sup>; bleomycin 7.5 to 15 mg intramuscularly given twice weekly; and doxorubicin 60 mg/m<sup>2</sup> intravenously given once per month.<sup>135,136</sup> Pegylated doxorubicin used in advanced MF has resulted in an overall response of 88 percent.<sup>137</sup> Purine analogues including fludarabine and pentostatin have response rates up to 50 percent.<sup>138-140</sup> Gemcitabine has a similar response rate.<sup>141</sup>

Neither single-agent nor multiagent therapy cures MF, and immunomodulatory or biological therapies are considered superior to cytotoxic agents.<sup>5,142</sup> Single-agent and multiagent chemotherapy result in earlier relapse, measured by shorter median time to next treatment (3.9 months) compared to interferon (8.7 months) and HDACi (4.5 months).<sup>143</sup> The survival of patients treated with aggressive multimodal chemotherapy is not different from the survival of patients treated conservatively, but aggressive chemotherapy results in greater toxicity, increased immunosuppression, and an increased risk of serious infections, leading to death in the majority of patients who develop this complication.<sup>144</sup> Multiagent chemotherapy is generally reserved as a last resort, for palliation in end-stage disease, or as a bridge to stem cell transplantation (SCT).<sup>6-8</sup>

**Combined Modality Therapy** Although not yet standard of care, several multidrug regimens utilizing immunomodulators and biologic agents reportedly improve clinical response in patients with MF, including combination of ECP with low-dose interferon alfa and oral bexarotene; prednisone and fludarabine; and PUVA and oral bexarotene.<sup>3,145</sup> SS patients achieved a 89 percent response rate when treated with a multimodal regimen of ECP in combination with two systemic biologic response modifiers: interferon alfa, interferon gamma, retinoids, PUVA, and/or sargramostim, a recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF) that functions as an immunostimulator.<sup>146</sup>

Because in general MF is an indolent malignancy of T cells with excellent prognosis in early stages, the treatment should be conservative, with skin-directed therapies (nitrogen mustard, topical glucocorticoids, topical bexarotene) combined with light therapy, low-dose interferon, low-dose methotrexate, or other single-agent chemotherapy.

The survival of patients treated with aggressive chemotherapy is not different from the survival of patients treated conservatively, but aggressive chemotherapy results in greater toxicity. Because no curative therapy exists, the goal of therapy is to prevent progression to more advanced stages and to preserve the patient's quality of life for as long as possible.

**Stem Cell Transplant** Patients with aggressive disease such as tumor-stage MF, transformed MF, or SS refractory to multiple lines of systemic therapies may be candidates for hematopoietic SCT. Allogeneic SCT may potentially cure or induce long-term remissions in patients via a graft-versus-lymphoma (GVL) effect.<sup>8</sup> However, allogeneic SCT carries a high risk of morbidity and mortality due to acute and chronic immune-mediated graft-versus-host disease (GVHD) and infections. Autologous SCT is not recommended for CTCL due to lack of durable response.<sup>146a</sup>

Prior to transplantation, myeloablative conditioning (MAC) provides maximal antitumor effect but has higher risk of toxicity and non-relapse mortality compared to reduced-intensity conditioning (RIC), which is more appropriate for older patients with comorbidities. MAC and RIC have similar rates of GVHD.<sup>146b</sup> More prospective studies are needed to determine optimal conditioning regimens and timing of allogeneic SCT.

## PROGNOSIS

Prognosis largely depends on the stage at presentation. Patients with T1 disease have a similar life expectancy as control populations, median survival for stage IB/IIA is about 12.9 years, and median survival for stage IV is 1.5 years.<sup>18,146c</sup> About a quarter of MF/SS patients die of their disease, and other deaths are attributed to cardiopulmonary events, pneumonia, and secondary malignancies.<sup>55</sup> A large outcomes study of advanced MF/SS patients identified four independent prognostic variables affecting survival: (1) stage IV disease, (2) age greater than 60, (3) elevated serum lactate dehydrogenase, and (4) large cell transformation. For patients with no or one of these risk factors, the 5-year overall survival rate is 67.8 percent, for two risk factors is 43.5 percent, and for three to four risk factors is 27.6 percent.<sup>146d</sup>

## CD30+ LYMPHOPROLIFERATIVE DISORDERS

Primary cutaneous CD30+ LPDs are the second most common CTCLs after MF and represent approximately 30 percent of CTCL cases.<sup>1</sup> CD30+ LPDs represent a spectrum of disease with LyP and PCALCL on either end. They are indolent, carry a favorable prognosis, and tend to regress spontaneously.<sup>147</sup>

## PRIMARY CUTANEOUS ANAPLASTIC LARGE CELL LYMPHOMA

CD30+ PCALCLs can occur at any age, with the peak incidence in patients in their 60s, with a slight male predominance.<sup>148,149</sup> PCALCL can occur anywhere on the body. The lesions are brownish to violaceous nodules or tumors, ranging in number from solitary (most commonly) to numerous with generalized involvement. They may regress spontaneously. Histopathologically, at least 75 percent of the large cells should express CD30. Most cases are CD4+, with loss of pan-T-cell markers CD2, CD3, and CD5. In rare cases, the cells are CD8+CD30+. In contrast to systemic anaplastic large cell lymphoma (ALCL), primary cutaneous large cell lymphoma is negative for CD15 and epithelial membrane antigen.<sup>151</sup> The descriptive term *anaplastic* could be omitted from the name of this lymphoma because these lymphomas may have an anaplastic, immunoblastic, or pleomorphic cell morphology. Regardless of pathologic type, these CD30+ large cell lymphomas have a similar clinical course, treatment, and prognosis.<sup>151a</sup> In addition, primary cutaneous large cell lymphoma usually does not express anaplastic lymphoma kinase-1 (ALK-1) or the t(2;5) chromosomal translocation found in nodal ALCL.<sup>152,153</sup> Presence of ALK-1 in cutaneous lesions without systemic involvement does not carry a worse prognosis.

## LYMPHOMATOID PAPULOSIS

LyP is the benign counterpart of PCALCL. It is characterized by crops of erythematous, dome-shaped papules or nodules that may ulcerate spontaneously. It regresses over a few months with minor sequelae such as scarring or atrophy (Fig. 14-7B). The three main histologic types of LyP are A, B, and C. The infiltrate usually is wedge shaped with ulcer formation. The large atypical cells of type A resemble immunoblasts of Reed-Sternberg cells. These cells are surrounded by neutrophils and eosinophils. Type B cells resemble MF, with lichenoid lymphocytic infiltration of cells with cerebriform nuclei and some epidermotropism. Type C cells resemble ALCL, with sheets of large CD30+ cells in the infiltrate. The histologic distinction between LyP and the corresponding condition may be difficult, and clinical correlation is required.<sup>154</sup> In rare cases, LyP evolves into more aggressive primary cutaneous large cell lymphoma. In addition, a higher incidence of lymphoid and nonlymphoid malignancies is observed in patients with LyP.<sup>155</sup> In some cases, distinction between LyP and PCALCL cannot be made because of discrepancies between clinical features and histologic appearance. These cases are referred to as *borderline lesions*, and their classification should take into consideration their clinical behavior and appearance (Fig. 14-7A).

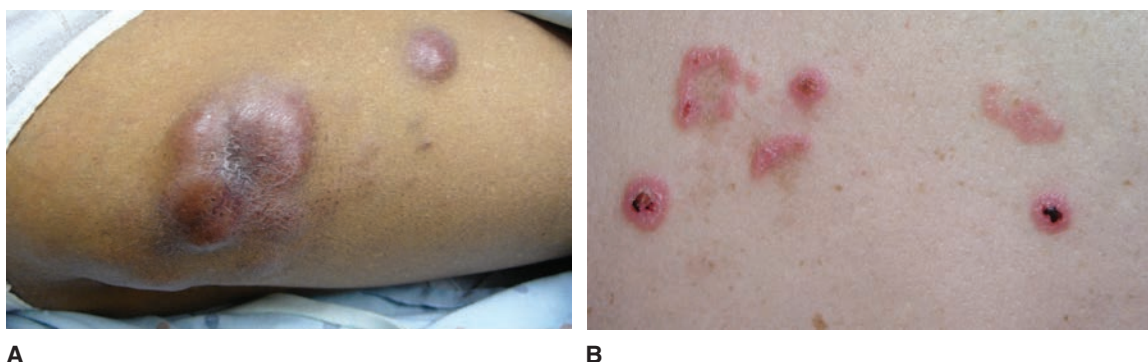


**Figure 14-7.** CD30+ lymphoproliferative disorders. **A.** Primary cutaneous anaplastic large cells lymphoma. Large cutaneous tumors on the anterior thigh. **B.** Lymphomatoid papulosis. Numerous small erythematous papules and small nodules. Some with necrotic centers in crops. Some lesions show spontaneous regression.



Other CD30+ LPDs include large cell transformation of MF, nodal ALCL, cutaneous NK/T-cell lymphoma, and Hodgkin lymphoma. While CD30+ LPDs have a favorable prognosis, transformed MF and nodal ALCL have poor prognoses. CD30+ LPDs may, in rare instances, progress to systemic involvement and have the same prognosis as nodal CD30+ lymphomas.

## TREATMENT

LyP is extremely responsive to low-dose methotrexate therapy, requiring 10 to 15 mg orally weekly, with noticeable clinical response within a month. Brentuximab vedotin was shown to be highly effective in patients with PCALCL and LyP (see “Monoclonal Antibody Conjugates”). Other treatment options include oral PUVA therapy, retinoids, topical and systemic glucocorticoids, and intralesional and systemic interferon- $\alpha$ .<sup>150,150a,154</sup> Treatment of primary cutaneous large cell lymphoma depends on the extent of skin involvement. In cases of solitary lesions, radiotherapy is often the best initial treatment modality. A combination of PUVA and interferon- $\alpha$  may be considered for more disseminated disease. Combination chemotherapy should be reserved for resistant cases.<sup>150,150a,156</sup>

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## CHAPTER 15

# MATURE T-CELL AND NATURAL KILLER CELL LYMPHOMAS

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### SUMMARY

The mature T-cell and natural killer (NK) cell lymphomas represent 10 to 15 percent of the non-Hodgkin lymphomas by incidence and comprise 23 clinicopathologic entities in the most recent classification. They include cutaneous T-cell lymphomas, discussed in Chap. 14, and systemic T-cell lymphomas, which are discussed here. The systemic T-cell lymphomas have highly variable courses and are typically aggressive and frequently less responsive to conventional chemotherapy than their B-cell counterparts. The most common systemic T-cell and NK cell lymphomas worldwide include peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), and angioimmunoblastic T-cell lymphoma (AITL), representing 26 percent and 19 percent of systemic T-cell and NK cell lymphomas, respectively. There is considerable geographic variation in the incidence of certain entities, such as adult T-cell leukemia/lymphoma (ATL) and extranodal NK/T-cell lymphoma (ENKTL). In view of the rarity of systemic T-cell and NK cell disorders, large randomized trials are lacking to guide therapies. Treatment strategies are generally based upon the best data available, which includes prospective phase II studies and retrospective analyses. The most frequently used regimens for the more common entities, PTCL-NOS, AITL, and anaplastic large cell lymphoma (ALCL), are cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) based, although long-term

**Acronyms and Abbreviations:** AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; ASCT, autologous stem cell transplantation; ATL, adult T-cell leukemia/lymphoma; BCCA, British Columbia Cancer Agency; CHOEP, cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone; CHOP, cyclophosphamide, hydroxydaunorubicin (doxorubicin), vincristine (Oncovin), prednisone; CR, complete response; CT, computed tomography; DHAP, dexamethasone, cytarabine, cisplatin; DSHNHL, German High-Grade Non-Hodgkin Lymphoma Study Group; EATL, enteropathy-associated intestinal T-cell lymphoma; EBV, Epstein-Barr virus; EFS, event-free survival; ENKTL, extranodal NK/T-cell lymphoma; FFS, failure-free survival; HTLV, human T-lymphotrophic virus; hyperCVAD, cyclophosphamide, vincristine, doxorubicin, methotrexate, cytarabine; ICE, ifosfamide, carboplatin, etoposide; IVAC, ifosfamide, etoposide, cytarabine; IPI, International Prognostic Index; IPTCLP, International Peripheral T-Cell Lymphoma Project; LDH, lactate dehydrogenase; MACOP-B, high-dose methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin; MEITL, monomorphic epithelioid intestinal T-cell lymphoma; NK, natural killer; ORR, overall response rate; OS, overall survival; PCR, polymerase chain reaction; PET, positron emission tomography; PFS, progression-free survival; PIT, prognostic index for T-cell lymphoma; PR, partial response; PTCL, peripheral T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; SMILE, dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide; TCR, T-cell receptor.

outcomes are often unsatisfactory. Therefore, ongoing clinical trials are aimed at improving upon CHOP by adding novel agents or using alternate regimens. Although controversial, patients are often considered for consolidation with autologous stem cell transplant in first remission to improve remission durations. Recently, targeted agents specific for particular T-cell and NK cell lymphomas, such as brentuximab vedotin for ALCL and crizotinib for anaplastic lymphoma kinase (ALK)-positive ALCL, are now allowing the investigation of more individualized therapy for these entities. Furthermore, for a considerable number of the T-cell and NK cell lymphoma entities, including ENKTL and ATL, CHOP-based therapy is ineffective, and treatment strategies are disease specific. There is still much to learn about the biology and potential drug targets for these diseases, and ongoing studies using gene-expression profiling and genomics may help answer some of these questions. In addition, it is hoped that ongoing clinical trials evaluating disease-specific treatment approaches and the use of novel and often targeted agents will lead to improved outcomes for patients with these diseases.

### OVERVIEW OF MATURE T-CELL LYMPHOMAS

The peripheral T-cell lymphomas (PTCLs) represent approximately 10 to 15 percent of non-Hodgkin lymphomas and are made up of 23 heterogeneous diseases (Table 15-1).<sup>1,1a</sup> The most common entities—peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS); angioimmunoblastic T-cell lymphoma (AITL); anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALCL); and ALK-negative ALCL—account for approximately 60 percent of cases. This overview primarily pertains to these most common subtypes of PTCL, and more detailed discussion of other subsets of PTCL follows this discussion.

As a result of the rarity of these disorders, there are no randomized controlled clinical trials to drive treatment decisions in PTCL. Our most comprehensive knowledge of the expected outcomes for patients with PTCL is mainly based upon three large retrospective series: the International Peripheral T-Cell Lymphoma Project (IPTCLP), the British Columbia Cancer Agency (BCCA) series, and a Swedish series. These series reported outcomes on 1314 cases, 199 cases, and 755 cases, respectively.<sup>2-4</sup> The prospective Comprehensive Oncology Measures for Peripheral T-Cell Lymphoma Treatment (COMPLETE) study is an ongoing registry of patients from the United States that has reported data on 253 subjects to date.<sup>5</sup> These registries underscore the geographical variations in the incidence of these disorders (Table 15-2).

### DIAGNOSIS OF PERIPHERAL T-CELL LYMPHOMA

The diagnosis of PTCL is based on histologic features, immunophenotype, molecular studies, and clinical presentation. While B-cell lymphomas are often characterized by a specific immunophenotypic profile, T-cell lymphomas are often characterized by antigen aberrancy that may vary within a subtype or even during the course of the disease.<sup>6,7</sup> In the IPTCLP, a consensus diagnosis (three of four expert pathologists arriving at the same diagnosis) was reached only 74 to 81 percent of the time for ALK-negative ALCL, PTCL-NOS, and AITL. Diagnoses were significantly refined in 154 out of 1314 cases when clinical information was available.<sup>3</sup> In establishing the diagnosis of T-cell non-Hodgkin lymphoma, it is important to exclude a reactive process, particularly when

**TABLE 15-1.** 2016 WHO Classification of Mature T-Cell and Natural Killer Cell Neoplasms (Excluding Primary Cutaneous Lymphomas)

|                                                                                                              |
|--------------------------------------------------------------------------------------------------------------|
| Peripheral T-cell lymphoma, NOS                                                                              |
| Angioimmunoblastic T-cell lymphoma                                                                           |
| Follicular T-cell lymphoma*                                                                                  |
| Anaplastic large cell lymphoma, ALK positive                                                                 |
| Anaplastic large cell lymphoma, ALK negative                                                                 |
| Breast implant–associated anaplastic large cell lymphoma*                                                    |
| Enteropathy-associated T-cell lymphoma                                                                       |
| Monomorphic epitheliotropic intestinal T-cell lymphoma                                                       |
| Adult T-cell leukemia/lymphoma                                                                               |
| Hydroa vacciniforme–like lymphoma                                                                            |
| T-cell prolymphocytic leukemia                                                                               |
| T-cell large granular lymphocytic leukemia                                                                   |
| Hepatosplenic T-cell lymphoma                                                                                |
| Extranodal NK/T-cell lymphoma, nasal type                                                                    |
| Aggressive NK cell leukemia                                                                                  |
| Systemic EBV+ T-cell lymphoproliferative disease of childhood (associated with chronic active EBV infection) |
| Chronic lymphoproliferative disorder of NK cells*                                                            |

ALK, anaplastic lymphoma kinase; EBV, Epstein-Barr virus; NK, natural killer; NOS, not otherwise specified.

\*Provisional entities.

the clinical picture is not congruent with the pathologic features, when the diagnostic biopsy is small, or when a clonal T-cell receptor (TCR) rearrangement is the primary or only reason for the diagnosis because reactive nonmalignant conditions often mimic PTCL.<sup>8-10</sup>

## INITIAL WORKUP

In addition to routine physical examination, initial evaluation should include systemic imaging (computed tomography [CT] of the chest, abdomen, and pelvis with contrast or positron emission tomography

[PET]/CT); marrow aspirate/biopsy; and laboratory evaluation (including complete blood count, lactate dehydrogenase or lactate dehydrogenase [LDH], comprehensive metabolic panel). Serologic testing for human T-cell lymphotropic virus (HTLV) 1 is particularly important in establishing a new diagnosis of PTCL in a person from an endemic area as adult T-cell leukemia/lymphoma (ATL) represents approximately 9 percent of PTCL, is associated with a different prognosis, and usually requires alternative therapy.<sup>3</sup>

## Evaluating Prognosis in Peripheral T-Cell Lymphoma

The International Prognostic Index (IPI) established for evaluating aggressive lymphomas has been effective in risk-stratifying patients with PTCL, although the utility in AITL is less clear (Table 15-3).<sup>11</sup> The “prognostic index for T-cell lymphoma (PIT)” is an improved index developed specifically for PTCL that includes age, performance status, LDH level, and marrow involvement.<sup>7,12</sup> Other prognostic indices, such as the IPTCLP score, have been suggested for PTCL. Each has some value, although none provides a significant improvement over IPI in terms of impacting clinical management.<sup>13</sup> It is important to note that even patients identified as low risk by these indices often experience disappointing outcomes. For example, in the IPTCLP, the 5-year failure-free survival (FFS) for patients with no or one IPI risk factor was only 33 percent for PTCL-NOS and 34 percent for AITL. For this reason, the approach to management of PTCL patients usually does not differ significantly based on IPI alone. Nevertheless, in patients with ALK-positive ALCL, which tends to be more responsive to chemotherapy, the progression-free survivals (PFSs) for patients with zero/one, two, three, and four/five IPI risk factors are 80 percent, 60 percent, 40 percent, and 25 percent, respectively.<sup>14</sup> Consequently, patients with higher risk ALK-positive ALCL may be treated similarly to those with the less favorable PTCL histologies.

## APPROACH TO INITIAL THERAPY

CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy remains the most commonly employed backbone for upfront therapy of PTCL, based on extrapolation from studies done in aggressive B-cell lymphomas. In the IPTCLP, more than 85 percent of patients received CHOP-based therapy, and in contrast to ALK-positive ALCL where the 5-year FFS was 60 percent, the 5-year FFSs for PTCL-NOS, AITL, and ALK-negative ALCL were only 20 percent, 18 percent, and 36 percent, respectively (Table 15-3). Similar outcomes were observed in the BCCA series, with 5-year PFSs of 29 percent, 13 percent, and 28 percent

**TABLE 15-2.** Incidence of Lymphoma Subtypes by Geographic Region

| Subtype       | Registry | PTCL-NOS | AITL | ALCL, ALK+ | ALCL, ALK– | NK/T | ATL | EATL |
|---------------|----------|----------|------|------------|------------|------|-----|------|
| North America | IPTCL    | 34%      | 16%  | 16%        | 8%         | 5%   | 2%  | 6%   |
|               | BCCA     | 59%      | 5%   | 6%         | 9%         | 9%   | NA* | 5%   |
|               | COMPLETE | 34%      | 15%  | 11%        | 8%         | 6%   | 2%  | 3%   |
| Europe        | IPTCL    | 34%      | 29%  | 6%         | 9%         | 4%   | 1%  | 9%   |
|               | Swedish  | 34%      | 14%  | 9%         | 15%        | 4%   | NA* | 9%   |
| Asia          | IPTCL    | 22%      | 18%  | 3%         | 3%         | 22%  | 25% | 2%   |

AITL, angioimmunoblastic T-cell lymphoma; ALCL ALK–, anaplastic large cell lymphoma anaplastic lymphoma kinase negative; ALCL ALK+, anaplastic large cell lymphoma anaplastic lymphoma kinase positive; ATL, adult T-cell leukemia/lymphoma; BCCA, British Columbia Cancer Agency; COMPLETE, Comprehensive Oncology Measures for Peripheral T-Cell Lymphoma Treatment; EATL, enteropathy-associated T-cell lymphoma; IPI, International Prognostic Index; IPTCL, International Peripheral T-Cell Lymphoma Project; NA, not available; NK/T, natural killer cell/T-cell lymphoma; NOS, not otherwise specified; PTCL, peripheral T-cell lymphoma.

\*ATL patients were excluded in both the BCCA and Swedish Registry Studies.



**TABLE 15–3.** Characteristics and Outcomes in Common Peripheral T-Cell Lymphoma Subtypes

|              |        |            | % IPI           |                 |                 | 5-Year OS by IPI |                  |                  |                  |
|--------------|--------|------------|-----------------|-----------------|-----------------|------------------|------------------|------------------|------------------|
| PTCL Subtype | Number | Median Age | 0–1             | 2–3             | 4–5             | 5-Year OS*       | 5-Year PFS*      | 0–1              | 4–5              |
| PTCL-NOS     |        |            |                 |                 |                 |                  |                  |                  |                  |
| IPTCL        | 229    | 60         | 28              | 57              | 15              | 32%              | 20%              | 50%              | 11%              |
| BCCA         | 117    | 64         | 30              | 47              | 22              | 35%              | 29%              | 64%              | 22%              |
| Swedish      | 256    | 69         | 17 <sup>†</sup> | 59 <sup>†</sup> | 24 <sup>†</sup> | 28%              | 21%              | NA               | NA               |
| AITL         |        |            |                 |                 |                 |                  |                  |                  |                  |
| IPTCL        | 213    | 65         | 14              | 59              | 28              | 32%              | 18%              | 56%              | 25%              |
| BCCA         | 10     | 66         | 0               | 30              | 70              | 36%              | 13%              | NA               | NA               |
| Swedish      | 104    | 70         | 4 <sup>†</sup>  | 69 <sup>†</sup> | 27 <sup>†</sup> | 31%              | 20%              | NA               | NR               |
| ALCL ALK–    |        |            |                 |                 |                 |                  |                  |                  |                  |
| IPTCL        | 72     | 58         | 41              | 44              | 15              | 49%              | 36%              | 74%              | 13%              |
| BCCA         | 18     | 55         | 44              | 22              | 33              | 34%              | 28% <sup>‡</sup> | 66% <sup>‡</sup> | 25% <sup>‡</sup> |
| Swedish      | 115    | 67         | 34              | 42              | 24              | 38%              | 31%              | NA               | NA               |
| ALCL ALK+    |        |            |                 |                 |                 |                  |                  |                  |                  |
| IPTCL        | 76     | 34         | 49              | 37              | 14              | 70%              | 60%              | 90%              | 33%              |
| BCCA         | 12     | 32         | 67              | 25              | 8               | 58%              | 28% <sup>‡</sup> | 66% <sup>‡</sup> | 25% <sup>‡</sup> |
| Swedish      | 68     | 41         | 55 <sup>†</sup> | 39 <sup>†</sup> | 6 <sup>†</sup>  | 79%              | 63%              | NA               | NA               |
| EATL         |        |            |                 |                 |                 |                  |                  |                  |                  |
| IPTCL        | 62     | 61         | 25              | 63              | 13              | 20%              | 4%               | 29%              | 15%              |
| BCCA         | 9      | 61         | 0               | 30              | 70              | 22%              | 22%              | NA               | NA               |
| Swedish      | 68     | 68         | 42              | 44              | 14              | 20%              | 18%              | NA               | NA               |
| NK/T         |        |            |                 |                 |                 |                  |                  |                  |                  |
| IPTCL        | 35     | 44         | 26              | 57              | 17              | 9%               | 6%               | 17%              | 20%              |
| Extranasal   | 92     | 52         | 51              | 47              | 2               | 42%              | 29%              | 57%              | 0%               |
| Nasal        | 17     | 47         | 47              | 24              | 29              | 24%              | 15%              | 38%              | 20%              |
| BCCA         | 33     | 62         | 33              | 63              | 4               | 21%              | 14%              | NA               | NA               |
| Swedish      |        |            |                 |                 |                 |                  |                  |                  |                  |

AITL, angioimmunoblastic T-cell lymphoma; ALCL ALK–, anaplastic large cell lymphoma anaplastic lymphoma kinase negative; ALCL ALK+, anaplastic large cell lymphoma anaplastic lymphoma kinase positive; BCCA, British Columbia Cancer Agency; EATL, enteropathy associated T-cell lymphoma; IPI, International Prognostic Index; IPTCL, International Peripheral T-Cell Lymphoma Project; NA, not available; NK/T, natural killer cell/T-cell lymphoma; NOS, not otherwise specified; OS, overall survival; PFS, progression-free survival; PTCL, peripheral T-cell lymphoma.

\*Data from international T-cell lymphoma project in which >85% of patients received an anthracycline-based regimen without upfront transplant.

<sup>†</sup>Distribution of patients with the given IPI scores is based on the number of patients for whom the score could be completely calculated.

<sup>‡</sup>BCCA ALCL reported as both ALK+ and ALK–.

for PTCL-NOS, AITL, and ALCL, respectively.<sup>2</sup> In the Swedish Registry study where 84 percent of patients were treated with CHOP-like therapy, 5-year PFSs were 21 percent, 20 percent, and 31 percent for PTCL-NOS, AITL, and ALK-negative ALCL, respectively, compared to 63 percent for ALK-positive ALCL.<sup>4</sup>

Several prospective clinical trials in PTCL are available to inform us on the expected response rate to CHOP. In a phase II study evaluating CHOP induction therapy followed by autologous stem cell transplantation (ASCT) for untreated PTCL, the overall response rate (ORR) to CHOP was 79 percent, with a complete response (CR) rate of 39 percent.<sup>15</sup> Similarly, a small phase III study of CHOP versus VIP-rABVD (etoposide, ifosfamide, cisplatin alternating with Adriamycin, bleomycin, vinblastine, and dacarbazine) showed no significant difference in outcomes, with an ORR of 70 percent and a CR rate of 35 percent.<sup>16</sup>

Although the majority of patients receive CHOP, there is currently no standard frontline treatment approach for PTCL, as there are no randomized data guiding a preferred approach. Many have sought to augment the efficacy of CHOP by adding agents to the CHOP backbone. Several phase II studies adding the anti-CD52 antibody alemtuzumab to CHOP demonstrated impressive CR rates of 65 to 71 percent. However, the addition of alemtuzumab conferred significant toxicity, including Jacob-Creutzfeldt virus encephalitis, invasive aspergillosis, *Pneumocystis carinii* pneumonia, sepsis, Epstein-Barr virus (EBV)-related lymphoma, and cytomegalovirus reactivation.<sup>17–19</sup> Similarly, in a phase II study of denileukin diftitox plus CHOP, the ORRs and CR rates were 65 percent and 55 percent, respectively; however, three deaths occurred following one cycle of therapy, and four other patients were taken off study because of toxicity.<sup>20</sup>

The German High-Grade Non-Hodgkin Lymphoma Study Group (DSHNHL) evaluated the addition of etoposide to the CHOP regimen. They analyzed patients with PTCL treated on seven different prospective phase II or phase III protocols with either CHOP or CHOEP (cyclophosphamide, doxorubicin, vincristine, etoposide, prednisone).<sup>21</sup> Younger patients (<60 years old) with a normal LDH exhibited better outcomes if treated with CHOEP than with CHOP, with 3-year event-free survival (EFS) of 75.4 and 51 percent, respectively, although no difference in overall survival (OS) was observed. The benefits were greatest in the more favorable ALK-positive ALCL subtype, but there was a trend toward improved EFS in favor of CHOEP in other subsets as well ( $p = 0.057$ ). However, the addition of etoposide led to excessive toxicity in elderly patients. The Nordic group adopted CHOEP induction for subjects younger than age 60 years in a prospective study evaluating upfront stem cell transplantation for PTCL.<sup>22</sup> In this phase II study, patients received biweekly CHOEP followed by ASCT for the responders. The ORR to CHOEP was 82 percent with a CR rate of 51 percent. Although one must be cautious when comparing results from different study populations, these results appear superior to those reported by Reimer and colleagues for patients treated with CHOP followed by ASCT, who achieved CR in only 39 percent of cases.<sup>15</sup>

There are no randomized trials assessing the controversial approach of performing ASCT in first remission for PTCL, although several prospective studies suggested the benefit of this strategy. The aforementioned Nordic study enrolled 160 patients with PTCL, including 39 percent with PTCL-NOS, 19 percent with ALK-negative ALCL, and 19 percent with AITL, while excluding ALK-positive ALCL.<sup>22</sup> Patients were treated with CHOEP for six cycles (etoposide was omitted for patients >60 years of age), and those in CR or partial response (PR) proceeded to high-dose therapy with carmustine, etoposide, cytarabine, and melphalan (or cyclophosphamide) and ASCT. By intent-to-treat analysis, 71 percent of patients underwent ASCT, and the 5-year OS and PFS were 51 percent and 44 percent, respectively. Reimer and colleagues conducted the second largest prospective study evaluating ASCT in first remission following CHOP, enrolling 83 patients.<sup>15</sup> A 3-year OS rate of 48 percent was observed by intent-to-treat analysis. For those who were transplanted (66 percent of patients enrolled), outcomes were considerably more favorable, with a 3-year OS of 71 percent. In a retrospective analysis performed at Memorial Sloan Kettering Cancer Center to evaluate patients treated with the intent to transplant in first remission, interim PET imaging was found to be the most powerful predictor of outcome. Of the 53 percent of patients who had a negative interim PET

scan after four cycles, 59 percent were progression free after 5 years, including 53 percent of those with IPI of 3 or greater.<sup>23</sup>

## APPROACH TO RELAPSED OR REFRACTORY THERAPY

There are no randomized data or a standard of care to guide treatment of patients with relapsed or refractory PTCL.<sup>24</sup> In the largest series of patients with PTCL-NOS, AITL, and ALCL treated from 1976 to 2010, those with relapsed or refractory disease who did not proceed to hematopoietic stem cell transplant demonstrated a median OS of 5.5 months.<sup>25</sup> However, the outlook for this patient population may improve as several new agents have been approved for this setting, including romidepsin, belinostat, and pralatrexate.<sup>27,28</sup> In addition, brentuximab vedotin is listed in the National Comprehensive Cancer Network (NCCN) compendium of appropriate therapeutic agents for CD30-positive T-cell lymphomas (Table 15-4).<sup>29-32</sup>

For patients who are transplantation eligible, allogeneic transplantation should be considered in the relapsed/refractory setting. Once a donor is identified, intensive salvage chemotherapy options should be considered, including ICE (ifosfamide, carboplatin, etoposide) or DHAP (dexamethasone, cytarabine, cisplatin), as they have a high potential to induce major remissions that will optimize outcomes after transplantation.<sup>29</sup> However, these regimens are generally only tolerated for three to four cycles and should be followed promptly by consolidation with transplantation. Both myeloablative and reduced-intensity allogeneic stem cell transplantation have demonstrated up to 60 percent 3-year PFS.<sup>36-38</sup> The role of ASCT in relapsed/refractory PTCL is controversial. Several series suggested that ASCT rarely results in long-term disease control of PTCL, with the exception of patients with ALCL.<sup>33,34</sup> Other series, however, including registry data from the Center for International Blood and Marrow Transplant Research, reported more salutary results for ASCT after relapse.<sup>35</sup>

For patients who are not transplantation eligible, the goals of treatment are palliative, and therapy should be geared toward maintaining quantity and quality of life. Options for treatment include romidepsin, belinostat, pralatrexate, gemcitabine, bendamustine, and alemtuzumab.<sup>25-28,39-41</sup> In addition, brentuximab vedotin should be considered as the first choice for relapsed CD30-expressing ALCL patients who have not previously received this agent.<sup>21-32</sup>

In view of the heterogeneity of PTCL, there is an increasing interest in individualizing therapy based on histology and other factors. For example, brentuximab vedotin, a CD30 antibody-drug conjugate,

**TABLE 15-4.** Overall Response Rate to Agents FDA Approved for Relapsed/Refractory Peripheral T-Cell Lymphoma

| Subtype  | Pralatrexate* | Romidepsin† | Belinostat‡ | Brentuximab Vedotin§,¶ |
|----------|---------------|-------------|-------------|------------------------|
| PTCL-NOS | 31%           | 29%         | 23%         | 33%                    |
| AITL     | 8%            | 30%         | 46%         | 50%                    |
| ALCL     | 29%           | 24%         | 15%         | 86%                    |

AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; PTCL, peripheral T-cell lymphoma; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified.

\*Data extrapolated from OA O'Connor, B Pro, L Pinter-Brown, et al.<sup>25</sup>

†Data extrapolated from B Coiffier, B Pro, HM Prince, et al.<sup>26</sup>

‡Data extrapolated from S Horwitz, W Jurczak, A Van Hoof, et al.<sup>28</sup> and OA O'Connor, T Masszi, KJ Savage, et al.<sup>27</sup>

§Data extrapolated from B Pro, R Advani, P Brice, et al.<sup>31</sup> and SM Horwitz, RH Advani, NL Bartlett, et al.<sup>31</sup>

¶Brentuximab vedotin is National Comprehensive Cancer Network (NCCN) Compendium listed for relapsed/refractory CD30+ PTCL.

induced responses in patient with relapsed or refractory ALCL as well as those with CD30-positive AITL and PTCL-NOS.<sup>31,32</sup> Similarly, crizotinib, an ALK inhibitor, demonstrated significant activity in a small number of patients with relapsed ALK-positive ALCL and is being further investigated.<sup>42–44</sup> The histone deacetylase (HDAC) inhibitors, such as belinostat and romidepsin, appear to have preferential activity and duration of response in patients with AITL (Table 15–4).<sup>26,28</sup>

Gene-expression profiling has identified molecular classifiers that improve classification and prognostication in ALK-negative ALCL, AITL, and PTCL-NOS.<sup>45–48</sup> Furthermore, additional translocations and recurrent mutations have been identified that may help better classify PTCLs and identify potential treatment targets. Next-generation sequencing has identified a novel translocation within ALK-negative ALCL, t(6;7),<sup>49</sup> which potentially identifies a unique entity within ALK-negative ALCL associated with a better prognosis.<sup>50</sup> This mutation typically leads to reduced expression of the *DUSP22* gene, which likely functions as a tumor suppressor. A translocation producing an *ITK-SYK* fusion gene, t(5;9), was initially found in a subset of PTCL-NOS cases.<sup>51</sup> SYK expression was subsequently evaluated in 141 PTCL cases by immunohistochemistry and found to be overexpressed in 94 percent, although the translocation was only detected in 39 percent.<sup>52</sup> These findings suggest a potential role for SYK inhibitors in these cases.<sup>53</sup> Mutations involving the *TET2* gene appear to be common in AITL as well as PTCL-NOS expressing T-follicular helper ( $T_{FH}$ ) cell markers; they are less frequent among the other PTCL-NOS cases (24 percent) and absent in ALCL.<sup>54</sup> *TET2* is involved in epigenetic control of transcription through DNA methylation, and inactivating mutations of this gene were first identified in myeloid malignancies. These mutations signify a biologic connection between AITL and PTCL-NOS with AITL features ( $T_{FH}$ -like PTCL-NOS) and suggest a role for hypomethylating agents.

## ● PERIPHERAL T-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED

### DEFINITION

PTCLs that do not fit into any of the currently recognized histopathologic categories are designated PTCL, not otherwise specified (PTCL-NOS; Chap. 7).

### EPIDEMIOLOGY

PTCL-NOS is the most common of the mature T-cell neoplasms, representing approximately 25 to 30 percent of the total cases of T-cell lymphoma in Western countries.<sup>55–57</sup> In the United States, there are approximately 0.4 cases per 100,000 adults.<sup>60</sup> In Asia, PTCL-NOS accounts for 20 to 25 percent of cases of mature T-cell neoplasms (Table 15–2).<sup>3,56</sup> The median age of diagnosis is 60 years, with a male predominance (2:1).<sup>3,7</sup>

### CLINICAL FINDINGS

In general, patients with PTCL-NOS have aggressive, rapidly growing disease. Diffuse lymphadenopathy, constitutional symptoms, extranodal involvement, an elevated LDH level, and advanced-stage disease are common.<sup>55,56</sup> Hepatomegaly and splenomegaly occur in 17 percent and 24 percent of patients, respectively. Stages I, II, III, and IV disease are observed in approximately 14 percent, 17 percent, 26 percent, and 43 percent of cases, respectively. The marrow is involved in approximately 20 percent of cases.<sup>2,4</sup> Pruritus, peripheral eosinophilia, hypercalcemia, and hemophagocytic syndrome may accompany PTCL.<sup>58,59</sup>

## LABORATORY FINDINGS

### Histopathology

The majority of cases arise in lymph nodes, which exhibit mixtures of small and large atypical lymphoid cells, typically admixed with an inflammatory background (Chap. 7).<sup>60</sup> The immunophenotype is typically that of a mature T cell expressing either a CD4 or CD8 phenotype, most commonly CD4, although CD4/CD8 double-positive and double-negative cases have been reported.<sup>6,61</sup> Malignant cells are often characterized by antigen aberrancy that may vary from one patient to another or even vary during the course of the disease within a single patient.<sup>6,7</sup> Deletion of one or more pan-T-cell antigens is frequently observed,<sup>62</sup> along with rearrangements of the TCR.

## TREATMENT AND PROGNOSIS

As discussed previously, patients with PTCL-NOS are most commonly treated with CHOP-based regimens, although there is no uniform standard of care for first-line treatment. Few prospective data exist for CHOP alone in PTCL, but based on clinical trials employing CHOP as a component of therapy, it appears that CHOP produces an ORR as high as 79 percent and a CR rate as high as 39 percent.<sup>15</sup> However, with CHOP alone long-term remissions are uncommon, with PFS rates of 20 to 30 percent seen in the larger retrospective series.<sup>2–4</sup> Efforts to improve the efficacy of first-line therapy by adding new agents to CHOP, such as alemtuzumab, bortezomib, or denileukin diftitox, have not clearly resulted in significant benefit.<sup>20,12,19</sup> The addition of etoposide to CHOP appears to provide some benefit over CHOP in more favorable young patients, according to a retrospective analysis of seven prospective studies conducted by the DSHNHL.<sup>21</sup> Moreover, in a prospective study of CHOEP followed by ASCT, the ORR to CHOEP was 82 percent, with 51 percent achieving a CR.<sup>22</sup> Newer agents approved in the relapsed setting, such as romidepsin and brentuximab vedotin, are currently being combined with CHOP-based regimens and compared to standard CHOP in ongoing phase III clinical trials.

Another strategy for improving frontline therapy for PTCL-NOS has been through consolidation with ASCT in first remission, as discussed in “Approach to Initial Therapy.” Even though there are no randomized trials of ASCT in first remission, three prospective phase II trials supported this approach.<sup>15,22,23</sup> The largest of these studies demonstrated 5-year OS and PFS of 47 percent and 38 percent, respectively, in patients with PTCL-NOS.<sup>22</sup>

Allogeneic transplantation provides long-term disease control for a select group of patients with relapsed PTCL-NOS. Although transplant-related mortality is significant, retrospective studies have demonstrated up to 61 percent OS at 2 years in some trials. Consequently, allogeneic transplantation is often considered for fit patients who fail frontline therapy.<sup>23,38,63</sup>

Several promising newer agents, including belinostat, bendamustine, gemcitabine, and alemtuzumab, have become available for the treatment of PTCL-NOS in the relapsed setting.<sup>27,39–41</sup> Pralatrexate, romidepsin, and belinostat are approved broadly for PTCL, with an approximately 20 to 30 percent ORR in large phase II studies (Table 15–4).<sup>26,68,69</sup>

Overall, the 5-year FFS for PTCL-NOS is 20 percent, with a 5-year OS of 32 percent in patients treated with CHOP-based therapy.<sup>58</sup> The IPI and PIT have been used to risk stratify patients with PTCL-NOS and estimate prognosis. For patients with PIT scores of 0, 1, 2, or 3 or greater, the 5-year FFSs are 34 percent, 22 percent, 13 percent, and 8 percent, respectively. For patients with IPI scores of 1 or less, 2, 3, or 4 or greater, 5-year FFSs are 36 percent, 18 percent, 15 percent, and 9 percent, respectively.<sup>7</sup> PIT scores have not been used to direct treatment decisions, however (Table 15–3).<sup>13,12</sup>



## ● ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

### DEFINITION

AITL was initially described as angioimmunoblastic lymphadenopathy with dysproteinemia in 1974. It is now known that these entities are identical.<sup>1,65</sup>

### EPIDEMIOLOGY

AITL represents approximately 1 percent of all cases of lymphoma and approximately 20 percent of all T-cell lymphomas.<sup>56</sup> International series showed that AITL represents a higher portion of T-cell lymphomas in Europe than North America or Asia.<sup>3</sup> The male-to-female ratio is approximately 1:1, and the median age at diagnosis is approximately 65 to 70 years.<sup>3,4,7</sup>

### CLINICAL FINDINGS

Common features at presentation include B symptoms (fever, drenching sweats, and weight loss); generalized lymphadenopathy; rash; polyclonal hypergammaglobulinemia; blood eosinophilia; and autoimmune hemolytic anemia (direct Coombs positive). Some patients experience waxing and waning of symptoms over several years before a diagnosis is made. Patients with AITL can develop diffuse large B-cell lymphoma, which evolves from EBV-positive B cells, which usually are present in the lymphoid infiltrate. Emergence of diffuse large B-cell lymphoma can occur concomitantly with AITL at the time of diagnosis or may be the primary histology at relapse; therefore, repeat biopsies are strongly encouraged whenever relapse is suspected in order to guide therapy.<sup>66,67</sup>

### LABORATORY FINDINGS

#### Histopathology

AITL is characterized histologically by effacement of the normal lymphoid architecture with a pleomorphic cellular infiltrate and proliferation of small arborizing blood vessels. Small lymphocytes, plasma cells, immunoblasts, histiocytes, and eosinophils infiltrate involved lymph nodes. As a result, the normal lymphoid architecture may be obliterated, with loss of germinal centers and extensive intranodal neovascularization and expansion of the follicular dendritic cell meshwork (CD21). Scattered EBV+ B cells are almost always present and reflect the accompanying immunodeficient state. The malignant cells are CD4+  $\alpha\beta$  T cells with TCR- $\beta$  and - $\gamma$  rearrangements<sup>67–69</sup> and express CD10, a marker typical for AITL, approximately 90 percent of the time.<sup>70</sup> Abnormal karyotypes involving the X chromosome, and chromosomes 1, 3, and 5 are frequently found in AITL. A complex karyotype is a negative prognostic factor for AITL.<sup>74</sup> The normal counterpart of AITL is suspected to be the follicular T-helper cell based on genomic profiling, which reveals overexpression of specific markers, including CXCL13 and PD-1.<sup>71,72</sup> A subset of PTCL-NOS exhibits a similar gene-expression profile, suggesting it may behave similarly.<sup>73</sup>

### TREATMENT AND PROGNOSIS

Most patients with AITL are treated with CHOP-based regimens as discussed in “Approach to Initial Therapy.” The CR rate is similar to the rate observed with PTCL-NOS (approximately 50 to 60 percent). Nonrandomized data support upfront ASCT in patients who achieve a CR to first-line therapy.<sup>22</sup> Rare patients may be managed with glucocorticoid monotherapy, which can induce remissions, although responses are rarely sustained.<sup>75</sup> Responses to low-dose methotrexate

and cyclosporine have also been reported.<sup>76–78</sup> There have been reports of responses to 5-azacytadine- and lenalidomide-based treatment approaches.<sup>79,80</sup> Finally, response rates as high as 50 percent have been reported in CD30-positive relapsed/refractory AITL treated with brentuximab vedotin.<sup>31</sup>

Both the IPI and the PIT have been used to risk stratify patients but have not been used to direct therapy (Table 15–3).<sup>13,12,81</sup> One group has reported that immunoglobulin A levels, age older than 60 years, more than one extranodal site of involvement, anemia, and thrombocytopenia may also be of prognostic value in this disease.<sup>81</sup>

## ● ANAPLASTIC LARGE CELL LYMPHOMA

### DEFINITION

ALCL is defined as a CD30+ peripheral T-cell neoplasm. The disease has been provisionally subdivided into those that are ALK positive (60 to 70 percent) or negative (30 to 40 percent).<sup>1,82,83</sup> In addition to the systemic form, there are primary cutaneous forms (Chap. 14), which generally behave in an indolent fashion, as well as an entity called breast implant-associated ALCL.<sup>84,85</sup>

### EPIDEMIOLOGY

ALCL accounts for 6 to 24 percent of all T-cell lymphomas.<sup>2–4</sup> However, the prevalence of ALCL varies by geographic region (Table 15–2). ALK-positive ALCL accounts for 16 percent, 6 percent, and 3 percent of PTCL in North America, Europe, and Asia, respectively.<sup>3</sup> The median age of those with ALK-positive ALCL is 30 to 40 years old. Ninety percent of children with ALCL are ALK positive.<sup>82,86</sup> In the United States and Asia, the ALK-positive phenotype is more common (ratio being 2:1 and 3:2, respectively), whereas in Europe the ALK-negative phenotype is more common (3:2).<sup>3</sup> Both diseases exhibit a slight male predominance. ALK-negative ALCL accounts for 8 percent, 9 percent, and 3 percent of PTCL in North America, Europe, and Asia, respectively.<sup>3</sup> The median age of presentation is 55 to 60 for ALK-negative ALCL.<sup>3,14,64</sup>

### CLINICAL FEATURES

ALCL has an aggressive clinical course, frequently presenting with systemic symptoms, advanced disease, and extranodal localization. Patients with ALK-positive ALCL tend to be younger and have better performance statuses and lower serum LDH levels. Approximately 8 to 12 percent of patients with ALK-positive ALCL have morphologic evidence of marrow involvement.<sup>2–4</sup> The rate of detection of marrow involvement doubles when immunohistochemical staining techniques with anti-CD30 and anti-ALK antibodies are used.<sup>87</sup>

Patients with ALK-negative ALCL tend to be older, with higher LDH values, and worse performance status than ALK-positive cases. Extranodal presentations are more common in the ALK-negative population<sup>14</sup> and include sites such as the marrow, liver, lung, and skin, but rarely the central nervous system. Marrow involvement occurs in 12 to 22 percent of cases of ALK-negative ALCL.<sup>2–4</sup>

Breast implant-associated ALCL is a new clinical variant recognized as a provisional entity in the 2016 revised World Health Organization classification of lymphoid neoplasms.<sup>1a</sup> This disorder is characterized by ALK-negative ALCL associated with textured saline and silicone breast implants. The vast majority of patients present with swelling of the breast associated with a seroma. They present with an effusion within the breast and less frequently with a distinct breast mass.<sup>84,88–91</sup> In the largest series to date (87 patients), most presented with stage IE disease limited to the breast (83 percent), whereas 13.8 percent presented with stage II disease. Ninety-six percent of patients who

had been treated with complete surgical excision of the implant and capsule remained disease free at 5 years. For those treated with less-than-complete surgical resection, 11 percent remained disease free at 3 years.<sup>91</sup> Cases of stage IV disease are rare but have been reported.<sup>84</sup>

## LABORATORY FEATURES

ALCL cells tend to grow cohesively and are found preferentially invading lymph node sinuses.<sup>92</sup> There are three morphologic variants based on the size of the neoplastic and admixed reactive cells<sup>93</sup>: The “common type,” representing most cases, is characterized by large pleomorphic tumor cells; the “small cell variant,” representing 5 to 10 percent of cases, has a dominant population of small- to medium-size tumor cells mixed with large anaplastic cells that stain for CD30 and ALK; and the “lymphohistiocytic variant,” representing 5 to 10 percent of cases and is closely related to the small cell variant and contains small neoplastic cells mixed with large anaplastic cells and a large number of histiocytes (Chap. 7).<sup>94</sup>

ALK-positive ALCL is characterized by a nonrandom t(2;5) (p23;q35) translocation resulting in fusion of the *NPM* and *ALK* genes.<sup>95</sup> The resultant *NPM-ALK* fusion gene encodes an 80-kDa chimeric protein NPM-ALK (p80) that functions as an oncogene in ALK-positive ALCL. Of note, other translocations resulting in ALK expression in ALK-positive ALCL have also been identified but are rarer.<sup>49,96–102</sup> Thus, ALK immunoreactivity is a highly specific marker for this disease.<sup>82,83,96</sup> Either T or null immunophenotypes may be observed in ALCL. The T-cell variant expresses pan-T antigens CD2, CD3, CD4, CD5, and CD7, whereas the null variant lacks both T-cell and B-cell antigens, but usually expresses cytotoxic molecules such as granzyme B and perforin and has rearranged TCR genes, suggesting a T-cell origin.<sup>102,103</sup>

Genomic and proteomic cluster analyses strongly suggest that ALK-positive and ALK-negative ALCL are two distinct disease entities. *CEBPB*, *PTPN12*, *SERPINA1*, and *BCL6* genes are typically overexpressed in ALK-positive ALCL, and *CCR7*, *CNTFR*, interleukin (IL) 21, and IL-22 are overexpressed in ALK-negative ALCL.<sup>104</sup> Based on comparative genomic hybridization and fluorescence *in situ* hybridization for TP53 and ATM loci, gains of 17p and 17q24, and losses of 4q13–q21 and 11q14 are more common in ALK-positive tumors, whereas gains of 1q and 6p21 are preferentially observed in ALK-negative tumors.<sup>111</sup> These differences are underscored by the different responses of these two entities to therapy.<sup>86</sup> Interestingly, cases of ALK-negative ALCL that express DUSP22 appear to have a similar prognosis as ALK-positive ALCL, while those with TP63 expression are associated with especially poor prognosis.<sup>50</sup>

## TREATMENT AND PROGNOSIS

ALK-positive ALCL is the most chemosensitive of the T-cell lymphomas, with rates of survival and response similar to diffuse large B-cell lymphomas. ALCL is commonly seen in the pediatric age group, where intensive anthracycline-based chemotherapy regimens have been studied.<sup>105,106</sup> Approximately 90 percent of patients with ALK-positive ALCL treated with anthracycline-based chemotherapy achieve a tumor response, with 65 to 75 percent of pediatric patients remaining relapse free after 5 years.<sup>73,107</sup> Among adults, treatment with CHOP-based therapy has remained the most commonly used approach as more intensive regimens, such as high-dose methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin (MACOP-B) and doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone have not shown superiority to CHOP-based therapy.<sup>64,108</sup>

The IPI appears to be particularly helpful in risk stratification of ALK-positive ALCL, with some authorities suggesting that patients with high-risk ALK-positive ALCL should be treated similarly to those

with other forms of PTCL (Table 15–3).<sup>2,3,109</sup> The DSHNHL concluded in a retrospective assessment of seven phase II and phase III trials that patients with ALK-positive ALCL who were 60 years of age or younger and had a normal LDH had an improved EFS but not OS if treated with CHOP plus etoposide, rather than CHOP alone.<sup>21</sup> The Groupe d'Étude des Lymphomes de l'Adulte (GELA) group has suggested that the prognostic significance of ALK expression is limited to those older than age 40 years.<sup>64</sup> After relapse, it appears that patients can be cured with intensive salvage therapy (including ASCT) at a higher rate compared to other T-cell lymphomas.

Brentuximab vedotin, which combines an anti-CD30 antibody with monomethylauristatin E (MMAE) has demonstrated objective responses in more than 80 percent of patients with relapsed/refractory ALCL treated in a single-agent phase II trial.<sup>31</sup> The FDA approved brentuximab vedotin for the treatment of relapsed ALCL in 2011.<sup>110</sup> Brentuximab vedotin in combination with chemotherapy is being explored as a first-line therapy in ALCL. Crizotinib, an inhibitor of ALK tyrosine kinase that is FDA approved for the treatment of ALK-positive non-small cell lung cancer, has demonstrated encouraging responses in small series of ALK-positive ALCL, leading to ongoing trials in relapsed/refractory ALCL.<sup>43,44,111</sup>

ALK-negative ALCL typically presents with unfavorable features, including stages III and IV disease, B symptoms, high IPI scores, high LDH serum levels, and expression of TP63.<sup>50,113,114</sup> The disease typically pursues an aggressive clinical course and is less responsive to chemotherapy than ALK-positive ALCL. It has been common practice to treat patients with CHOP-like therapy, as with other forms of PTCL, as discussed in “Approach to Initial Therapy.” Similar to PTCL-NOS, consolidation with ASCT in first remission is often considered for ALK-negative ALCL based upon large prospective studies suggesting improved remission durations.<sup>15,22</sup> The IPTCLP reported 5-year FFS and OS rates of 36 percent and 49 percent, respectively, for patients with ALK-negative ALCL (Table 15–3).<sup>3</sup> CD56 expression has been shown to be an independent favorable prognostic factor for ALK-negative ALCL, as has expression of DUSP22.<sup>50,112</sup>

## ENTEROPATHY-ASSOCIATED T-CELL LYMPHOMA

### DEFINITION

Enteropathy-associated intestinal T-cell lymphoma (EATL) is a mature T-cell lymphoma that presents within the gastrointestinal tract. In 2016, the World Health Organization reclassified the intestinal T-cell lymphomas into two entities: EATL (previously referred to as type I EATL) and monomorphic epitheliotropic intestinal T-cell lymphoma (MEITL, previously referred to as EATL type II), which are differentiated based on their histopathology.<sup>1a,115</sup>

### EPIDEMIOLOGY

EATL constitutes approximately 2 to 10 percent of all PTCLs, and incidence varies by geographic distribution.<sup>2–4</sup> The median patient age at diagnosis is 55 to 65 years, with a slight male predisposition.<sup>115,116</sup> EATL comprises 60 to 80 percent of cases,<sup>115</sup> is most common in patients who have underlying celiac disease, and is strongly associated with the human leukocyte antigen (HLA)–DQ2 haplotype. Among European patients with EATL, 80 percent have type I EATL.<sup>115,117</sup> Patients who have refractory celiac disease, which does not improve with a gluten-free diet, have a significantly higher risk of EATL. In particular, those with refractory celiac disease who demonstrate a clonal expansion of abnormal

intraepithelial lymphocytes lacking CD3, CD8, and TCR markers, but express intracellular CD3, have a significantly higher risk of developing EATL.<sup>116</sup> In contrast, MEITL, present in 20 to 40 percent of cases, is less frequently associated with celiac sprue and the HLA-DQ2 haplotype.<sup>115,118</sup>

## CLINICAL FINDINGS

The majority of patients with EATL present with acute abdominal symptoms that often require urgent or emergent surgical procedures, resulting in diagnosis of the disease.<sup>119</sup> EATL typically presents with ulcerative lesions of the jejunum or ileum, which may perforate, though other regions of the gastrointestinal tract may also be affected. As a result of the malabsorption that accompanies the disease, frequent presenting signs and symptoms include weight loss, diarrhea, nausea, and vomiting, accompanied by abdominal pain and bowel obstruction.<sup>120</sup> The course can be fulminant; death may occur during treatment secondary to the consequences of intestinal perforation. Extraintestinal presentation of EATL is rare, and there is little data on the manifestations of cutaneous, neuromeningeal, or pulmonary presentations. Systemic symptoms should be considered signs of clinical progression, although they occur in less than 30 percent of patients with EATL.<sup>123,128</sup>

## LABORATORY FINDINGS

### Histopathology

The two types of EATL are recognized on the basis of the specific immunophenotype: EATL is characterized by CD56 negativity, and MEITL exhibits CD56 expression.<sup>118</sup> The histology of EATL type I demonstrates mostly medium-to-large tumor cells with round or angulated vesicular nuclei, prominent nucleoli, and pale-staining cytoplasm. There is often a moderate-to-abundant infiltrate of eosinophils, histiocytes, and small lymphocytes.<sup>122</sup> EATL characteristically demonstrates the following immunophenotype: positive for CD3, CD7, CD103, and usually CD30, but negative for CD4, CD5, CD8, and CD56. The cells also exhibit a cytotoxic phenotype and are positive for perforin, granzyme B, and TIA-1.<sup>123,124</sup> EATL METIL is characterized by a monomorphic infiltrate of small- to medium-size lymphocytes positive for CD3, CD8, CD56, and TCR- $\beta$  and negative for CD4. CD30 is often negative in EATL type 2.<sup>118</sup>

## TREATMENT AND PROGNOSIS

CHOP chemotherapy has been used most widely for both EATL and MEITL, with a 5-year relapse FFS of 4 to 22 percent and a 5-year OS of approximately 20 percent.<sup>2-4,115</sup> The addition of etoposide to CHOP does not appear to confer additional benefit.<sup>132</sup> Even patients who initially respond to CHOP therapy usually relapse after a median interval of 6 months, with a median survival after relapse of approximately 6 months to 1 year.<sup>12,115,120,125</sup> In patients who are transplantation eligible, high-dose chemotherapy followed by ASCT may improve outcomes.<sup>15,126,127</sup> The Scotland and Newcastle Group demonstrated that high-intensity therapy with CHOP alternating with IVE (ifosfamide, epirubicin, etoposide) and intermediate-dose methotrexate, followed by consolidation with ASCT resulted in a 5-year OS of 60 percent and PFS of 52 percent in the 50 percent of patients who were able to complete ASCT.<sup>119</sup> Many patients could not complete the course of chemotherapy, however, and there were a large number of complications, including gastrointestinal bleeding, small bowel perforation, and enterocolic fistulae. Twelve patients required either enteral or parental feeding.

Adverse prognostic factors include a history of celiac sprue, a large tumor mass ( $\geq 5$  cm) at diagnosis, an elevated LDH, and a nonambulatory performance status.<sup>115</sup> The PIT score predicts both OS and FFS in EATL better than the IPI.<sup>115</sup>

## ADULT T-CELL LEUKEMIA/LYMPHOMA

### DEFINITION

ATL is an uncommon lymphoproliferative neoplasm of mature CD4+CD25+ T cells caused by infection with the retrovirus HTLV-1, which was initially characterized in 1977.<sup>129,130</sup> These cells classically have a leukemic “flower-cell” appearance.<sup>131</sup> At least 5 percent of circulating abnormal T lymphocytes are required to diagnose ATL in patients without histologically proven tumor lesions.<sup>132</sup>

### EPIDEMIOLOGY

The incidence of ATL in the United States is approximately 0.05 cases per 100,000 people.<sup>133</sup> The disease prevalence parallels the geographic distribution of the HTLV-1 virus, with the highest incidences occurring in southern Japan, the Caribbean, Central and South America, intertropical Africa, Romania, and northern Iran (Table 15-2).<sup>134-138</sup> Among approximately 10 to 20 million HTLV-1 carriers, the lifetime risk of developing ATL is approximately 2.5 to 4 percent, with a mean latency of greater than 50 years.<sup>133,136,138,139</sup> HTLV-1 is transmitted through breastfeeding, blood products, and unprotected sexual intercourse. The overwhelming majority of ATL cases occur in patients infected during the early years of life.<sup>140</sup> In addition, the prolonged infection may increase chances of accruing subsequent mutations and ultimately malignant transformation. The mean age of patients with adult T-cell leukemia/lymphoma is 62 years, without a gender predominance.<sup>141,142</sup>

### CLINICAL FEATURES

Several clinical variants of ATL have been described: acute, lymphoma, chronic, and smoldering. These appear to have differing genomic alterations and different clinical courses.<sup>132,142,143</sup>

The acute variant of ATL represents 60 percent of cases and is characterized by patients who present with a leukemic presentation. An additional 20 percent of cases present with the lymphoma variant characterized by lymphadenopathy and less than 1 percent of leukemic cells in the blood. These subtypes exhibit an aggressive clinical course, with a median survival of less than 1 year. The majority of patients present with hepatosplenomegaly (50 percent of cases), lymphadenopathy, elevated LDH, hypercalcemia (50 percent of cases), and visceral and cutaneous lesions. The marrow is involved in approximately 35 percent of cases.<sup>142</sup> Most patients have generalized lymphadenopathy, particularly in the retroperitoneal and hilar regions, though the nodes are often relatively small and mediastinal masses are rare. Extranodal sites of disease include the lung, liver, skin, gastrointestinal tract, and central nervous system, including cord myelopathy and spastic paraparesis.

In contrast to the acute and lymphoma forms, the smoldering form of adult T-cell leukemia/lymphoma typically presents with a predominance of skin lesions or lung infiltration without visceral or marrow disease and minimal blood involvement ( $<5$  percent of lymphocytes). Patients with chronic ATL present with leukocytosis with lymphadenopathy and organomegaly without an elevated LDH or visceral involvement. Although smoldering and chronic ATL are characterized initially by indolent courses, the prognosis remains poor, with a survival of 4.1 years and 49 percent progressing to acute ATL after a median of 18.8 months.<sup>144</sup>

Opportunistic infections are common in patients with adult T-cell leukemia/lymphoma, even indolent forms,<sup>145</sup> including *P. carinii*, strongyloides, and cryptococcal meningitis, as well as bacterial and other fungal infections.



## LABORATORY FEATURES

### Histopathology

The neoplastic cells are pleomorphic, have highly lobulated nuclei (“clover leaf” or “flower-cell” appearances) with condensed nuclear chromatin, inconspicuous nucleoli, and a mature helper T-lymphocyte immunophenotype.<sup>131</sup> At least 5 percent of circulating abnormal T lymphocytes are required to diagnose ATL in patients without histologically proven tumor lesions.<sup>132</sup> In approximately 20 percent of cases, nuclear lobulation is less pronounced, and the cells may be difficult to distinguish from Sézary cells. These cells express the surface T-cell lymphocytic markers CD2, CD4 and CD5, CD45RO, CD29, and TCR- $\alpha\beta$ , are usually negative for CD7, CD8, and CD26, and show reduced CD3 expression. The lymphocytic activation markers HLA-DR, -DQ, and -DP and IL-2R $\alpha$  (CD25) are always present, whereas terminal deoxynucleotidyl transferase is typically absent.<sup>146</sup> Rare immunophenotypic variants (CD4-negative, CD8-positive, double-positive, or double-negative variants) have also been reported.<sup>147–149</sup> Although there are no specific chromosomal abnormalities diagnostic of ATL, the karyotype of ATL cells is usually complex in the aggressive variants of the disorder.<sup>130</sup> Clonal rearrangements of the TCR genes are typically present.<sup>150–152</sup>

### Laboratory Analysis

Patients with aggressive forms of ATL commonly present with an elevated LDH and hypercalcemia.

## TREATMENT AND PROGNOSIS

Overall, prognosis in ATL remains poor, with a median survival of less than 1 year for patients with aggressive subtypes. A multivariate analysis of 126 patients with acute (13 percent) and lymphoma (87 percent) subtypes of ATL performed by the IPTCLP indicated that the IPI was the only independent predictor of OS.<sup>141</sup> Although smoldering and chronic ATL are characterized initially by indolent courses, the prognosis remains guarded, with 5-year survivals of 40 percent and 50 percent, respectively; 49 percent of patients with the more indolent forms of ATL progress to acute ATL after a median time of 18.8 months.<sup>144</sup>

Despite its limited efficacy, cytotoxic chemotherapy remains the mainstay of therapy for this disease. A Japanese cooperative group developed a multidrug regimen called LSG15, which consists of seven cycles of VCAP (vincristine, cyclophosphamide, doxorubicin, and prednisone); AMP (doxorubicin, ranimustine, and prednisone); and VECF (vindesine, etoposide, carboplatin, and prednisone). LSG15 was evaluated in a phase II study that enrolled 96 patients with treatment-naïve acute type ( $n = 58$ ), lymphoma type ( $n = 28$ ), or unfavorable chronic type ( $n = 10$ ) ATL. The ORR was 81 percent, with 35 percent CRs and 45 percent PRs.<sup>153</sup> In view of these results, a phase III trial of LSG15 versus biweekly CHOP was performed in a similar patient population and demonstrated a superior CR rate (40 percent vs. 25 percent) and 3-year OS (24 percent vs. 13 percent), favoring the intensive arm; however, the median survival was only 13 months in the LSG15 arm.<sup>154</sup> These results highlight the inadequacy of CHOP in this disease, but the results seen with LSG15 leave significant room for improvement as well.

The role of antiviral therapy remains controversial. A recent meta-analysis of 254 patients with ATL demonstrated that there appears to be a benefit to first-line antiviral therapy, including interferon for patients with acute, chronic, and smoldering ATL, whereas patients with the lymphoma variant did not benefit. In patients with chronic and smoldering ATL treated with first-line antiviral therapy in combination with either chemotherapy or interferon, a 100 percent 5-year survival has been reported. In patients with leukemic ATL who were treated upfront with combined antiviral therapy and chemotherapy a 5-year OS

of 28 percent was observed, compared to 10 percent in patients treated with first-line chemotherapy alone. Maintenance antiviral therapy also was reported to confer an improved OS in patients treated with first-line chemotherapy. These findings have not yet been prospectively validated, however.<sup>155</sup>

Additionally, mogamulizumab (anti-CCR4 monoclonal antibody) has been approved in Japan for the treatment of relapsed or refractory adult T-cell leukemia/lymphoma. In a multicenter phase II study of 28 patients with relapsed/refractory ATL, the ORR with mogamulizumab was 50 percent, with a median OS of 13.7 months.<sup>156</sup> A randomized phase II study of modified LSG15 with or without mogamulizumab confirmed that the combination was well tolerated and had a CR rate of 52 percent compared to 33 percent with LGS15 alone.<sup>157</sup>

A large nationwide Japanese retrospective report of 386 patients with ATL assessed the role of allogeneic hematopoietic stem cell transplantation in ATL and demonstrated a 3-year OS for the entire cohort of 33 percent. Among patients who underwent related donor transplantation, donor HTLV-1 seropositivity adversely affected disease-associated mortality.<sup>158</sup> In patients who are transplantation eligible, allogeneic transplantation is therefore an attractive option, but ASCT has been found to be ineffective in ATL.<sup>159,160</sup>

In addition to combination chemotherapy used for other T-cell lymphomas in the relapsed or refractory setting, there is increased evidence regarding the usefulness of lenalidomide in ATL as well. A phase II Japanese study has shown a 42 percent ORR to single-agent lenalidomide.<sup>161</sup>

## HEPATOSPLENIC T-CELL LYMPHOMA

### DEFINITION

Hepatosplenic T-cell lymphoma (HSTCL) is a rare lymphoma that infiltrates the spleen, liver, and marrow. In the majority of cases, cells consist of mature  $\gamma/\delta$  T cells; however,  $\alpha/\beta$  HSTCL has also been reported.<sup>1</sup>

### EPIDEMIOLOGY

HSTCL is a rare lymphoma representing 3 percent of all T-cell lymphomas.<sup>162</sup> This disease typically occurs in young males at a median age of 35 years.<sup>7,163</sup> Immunosuppression following solid-organ transplantation or from use of anti-tumor necrosis factor- $\alpha$  or thiopurine agents (as are used for Crohn disease and other autoimmune diseases) has been implicated as a risk factor for this disease.<sup>164,165</sup>

### CLINICAL FINDINGS

Patients commonly present with isolated hepatosplenomegaly without lymphadenopathy, frequently accompanied by cytopenias, B symptoms, and an elevated serum LDH.<sup>56,163</sup>

## LABORATORY FINDINGS

### Histopathology

Neoplastic cells localize to sinusoids in the spleen, liver, and marrow. The malignant lymphocytes typically express CD3, CD56, and TCR- $\delta$ , but are negative for CD4 and usually CD8.<sup>166</sup> Clonal rearrangement of the TCR- $\gamma$  gene usually is present, and in most cases the lymphoma cells have an isochromosome 7q [17(q10)] along with trisomy 8, which also may be seen in the  $\alpha\beta$  variant of this disease.<sup>167–169</sup>

## TREATMENT AND PROGNOSIS

HSTCL is characterized by an aggressive course, with a median survival of 16 months.<sup>170</sup> The optimal therapy for HSTCL is not known. Small patient series and anecdotal reports described limited responsiveness and poor survival with CHOP and suggested better outcomes with other non-cross-resistant regimens such as ICE, hyperCVAD (cyclophosphamide, vincristine, doxorubicin, methotrexate, cytarabine), or IVAC (ifosfamide, etoposide, cytarabine).<sup>162,163,170–172</sup> Successful treatment with pentostatin has been reported as well.<sup>180</sup> Consolidation with either allogeneic stem cell transplantation or ASCT is likely necessary to achieve long-term remission.<sup>171</sup>

## ● EXTRANODAL NATURAL KILLER/T-CELL LYMPHOMA

### DEFINITION

Extranodal natural killer (NK)/T-cell lymphoma (ENKTL), nasal type, previously known as lethal midline granuloma, malignant granuloma, or angiocentric lymphoma, is an uncommon subtype of T-cell lymphoma.

### EPIDEMIOLOGY

ENKTL represents approximately 2 to 9 percent of T-cell lymphomas.<sup>2–4,174–175</sup> The disease typically afflicts middle-aged men, with a median age of 50 years at diagnosis, but may also affect children.<sup>133,134</sup> ENKTL occurs worldwide, with a strong geographic predilection for Asian populations from China, Japan, Korea, and Southeast Asia<sup>177</sup> and for Central and South American populations from Mexico,<sup>178</sup> Peru,<sup>179</sup> Argentina, and Brazil,<sup>180</sup> constituting 5 percent to 15 percent of lymphomas in these countries. Occasional case series have also been reported from Europe and North America (Table 15–2).<sup>181</sup>

### CLINICAL FEATURES

ENKTLs are almost exclusively extranodal.<sup>177</sup> Initial sites involved are often the nose and nasopharynx and occasionally the paranasal sinuses, tonsil, Waldeyer ring, and oropharynx. When nasal lymphomas destroy the floor of the nasal cavity, a characteristic hard-palate perforation is found.<sup>182</sup> Although they are usually localized, ENKTLs may disseminate to the skin, salivary glands, testis, and gastrointestinal tract. Interestingly, the lymphoma occasionally presents primarily in these sites without an apparent nasal primary. These “nonnasal” ENKTLs were thought to be more aggressive.<sup>183</sup> Modern imaging technology, such as PET/CT, shows that most, if not all, nonnasal lymphomas are associated with occult nasal primaries and implies that they are disseminated nasal lymphomas.<sup>184,185</sup> With improved treatment strategies, primary sites of presentation are no longer an independent prognostic indicator.<sup>186</sup> Rarely, the lymphoma can evolve into NK cell leukemia, which is characterized by widespread systemic dissemination and involvement of the marrow and blood.

### LABORATORY FINDINGS

#### Histopathology

The histopathology shows angiocentric plasmomorphous small- or medium-size atypical lymphoid cells with vascular invasion and ischemic tissue necrosis. Marrow hemophagocytosis may be present. As NK and T cells share a common ontogeny, the malignant cells express CD2 and CD7 but are negative for surface CD3. They also express CD16, CD56, cytoplasmic CD3ε, and CD57 and often demonstrate clonal

rearrangements in the TCR genes.<sup>1,175,182</sup> Neoplastic cells are invariably infected by EBV, which is detected most reliably by *in situ* hybridization for EBV-encoded RNA (EBER), a diagnostic requisite.<sup>177</sup> Rare cases may not express CD56.<sup>1</sup>

#### Laboratory Assessments

EBV DNA polymerase chain reaction (PCR) measured in plasma has been found to correlate with tumor burden, and serial EBV PCR monitoring is useful for assessing responses and disease recurrence.<sup>187,188</sup>

## TREATMENT AND PROGNOSIS

Outcomes of localized NK/T-cell lymphoma are best with combined chemotherapy and radiation therapy. In studies of radiation therapy alone, 75 to 100 percent of patients respond; however, systemic relapse rates are as high as 25 to 40 percent.<sup>189,190</sup> Previously, patients were treated with CHOP-based therapy in combination with radiation with a CR rate of 59 percent and 3-year disease-free survival of 25 percent.<sup>191–193</sup>

L-Asparaginase has a major single-agent activity in NK/T-cell lymphomas, and is incorporated into most modern regimens for this disease.<sup>194</sup> L-Asparaginase combined with gemcitabine, oxaliplatin, and radiation therapy exhibits an ORR of 96 percent and a local and systemic relapse rate of 10 to 15 percent in a phase I study.<sup>195</sup> Vincristine and prednisolone in combination with L-asparaginase produce an ORR of 89 percent when combined with radiation therapy. SMILE (dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide) in combination with radiation therapy demonstrates an 82 percent response rate with a CR rate of 78 percent.<sup>186</sup> L-Asparaginase has also been studied in combination with methotrexate and dexamethasone, with an ORR of 78 percent.<sup>197</sup> The combination of gemcitabine, oxaliplatin, and L-asparaginase (GELOX) in combination with radiation therapy results in a response rate of up to 96 percent.<sup>197a</sup> In patients with localized ENKTL, it is standard to consolidate asparaginase-based chemotherapy with radiation therapy. In the setting of patients with advanced-stage or relapsed/refractory disease, combination chemotherapy remains the standard treatment.<sup>182</sup> Studies of SMILE demonstrated an ORR of 25 to 80 percent in disseminated disease.<sup>186,196</sup> NK cell leukemia is characterized by widespread systemic dissemination and involvement of the marrow and blood and is associated with an extremely poor survival, measured only in weeks. Case series using checkpoint inhibitors demonstrate CRs in patients with relapse after L-asparaginase-based therapy and are promising.<sup>197b</sup>

EBV DNA PCR measured in the plasma has been found to correlate with tumor burden, and serial EBV PCR monitoring is useful for assessing responses and disease recurrence.<sup>187,188</sup> In view of the improved efficacy of L-asparaginase-based regimens, the role of ASCT remains unclear.

Even though the IPI has been found to be effective in risk stratification of ENKTL, Lee and colleagues have developed an NK/T-cell lymphoma-specific prognostic index that also predicts prognosis (Table 15–3).<sup>2,3,186</sup> This model includes B symptoms, elevated LDH, stage, and presence of regional lymph nodes. Those with no risk factors, one risk factor, two risk factors, and three or more risk factors had a 5-year OS of 81 percent, 64 percent, 34 percent, and 4 percent, respectively.<sup>198</sup>

## SUBCUTANEOUS PANNICULITIS-LIKE T-CELL LYMPHOMA

### Definition

Subcutaneous panniculitis-like T-cell lymphoma (SPTL) is a primary cutaneous T-cell lymphoma presenting with painful subcutaneous nodules.<sup>199–202</sup> The lesions consist of atypical lymphoid cells and reactive

histiocytes with admixed adipose tissue often associated with coagulation necrosis. Histologically, the cells express an  $\alpha/\beta$  phenotype. The  $\gamma/\delta$  phenotype of this disease is now classified as cutaneous  $\gamma/\delta$  T-cell lymphoma.<sup>1,202</sup>

### Epidemiology

As discussed in a worldwide retrospective analysis, SPTL is a rare disorder accounting for 0.9 percent all T-cell lymphomas.<sup>7,203</sup> SPTL is primarily a disorder of adults, with an average age at diagnosis in the mid- to late 30s, although cases have also been reported in children.<sup>204</sup> SPTL has a female predominance, with a male-to-female ratio of 0.5.<sup>204</sup>

### Clinical Features

Patients present with subcutaneous nodules that typically begin in the extremities and may spontaneously regress for a number of years, but eventually progress.<sup>205</sup> They may ulcerate, and patients may have systemic symptoms.

### Laboratory Features

The lesions consist of atypical lymphoid cells and reactive histiocytes with admixed adipose tissue often associated with coagulation necrosis. In most cases, the tumor is composed of mature CD8+  $\alpha\beta$  cytotoxic T cells that express TIA-1, granzymes, and perforin genes.<sup>202,204,205</sup>

### Treatment and Prognosis

Responses to combination chemotherapy have been reported but are usually of short duration.<sup>204–207</sup> Responses to glucocorticoids, interferon- $\alpha$ , zidovudine, and cyclosporine also have been reported.<sup>206,208,209</sup> Therapy for this disease remains controversial. Although standard chemotherapy may be effective, CRs are rare. Single-patient cases of successful allogeneic stem cell transplantation have been reported, but the rarity of this disease hampers further investigation of this modality.<sup>210,211</sup> The use of denileukin diftitox in two patients has been reported with evidence of activity, and bexarotene restored a clinical response in one of the patients after disease progression.<sup>212</sup> Single-agent bexarotene has also been shown to have significant clinical activity, with an ORR of 82 percent.<sup>213</sup>

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## CHAPTER 16

# PLASMA CELL NEOPLASMS: GENERAL CONSIDERATIONS

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### SUMMARY

Plasma cell neoplasms are tumors derived from an expansion of mutated mature B-cells and their precursors. These neoplasms include essential monoclonal gammopathy (synonym: monoclonal gammopathy of unknown significance; Chap. 17), smoldering myeloma (Chap. 18), myeloma (Chap. 18), solitary and extramedullary plasmacytomas (Chap. 18), light-chain amyloidosis (Chap. 19), and Waldenström macroglobulinemia (Chap. 20). The prototype of a malignant plasma cell neoplasm is myeloma, which is characterized by complex genetic alterations, best assessed by metaphase cytogenetics, fluorescence *in situ* hybridization analysis, and gene-expression profiling. The genetic changes are more akin to solid tumors than to hematologic malignancies. Interactions between myeloma cells and the marrow microenvironment affect the survival, proliferation, and drug resistance of myeloma cells and the development of osteoporosis or osteolysis, which is a hallmark of myeloma. As in most malignancies, a cancer stem cell (e.g., myeloma stem cell) has been identified and is the most likely site of drug resistance, which almost invariably develops during treatment; such cells are not affected by the typical drugs one

uses in patients with myeloma. The best prognostic markers in myeloma in order of importance are the presence of (1) specific cytogenetic abnormalities, (2) extent of the disease by appropriate imaging techniques, such as magnetic resonance imaging and/or combined positron emission and computed tomographic imaging, (3) the serum-free light-chain level and kappa-to-lambda ratio, and (4) the use of the International Staging System. The development of several classes of drugs over the past decade in combination with transplantation has improved therapeutic outcomes significantly in patients achieving an unequivocal complete remission. Thus, optimal techniques to assess minimal residual disease have also become important.

### DEFINITION AND HISTORY

Plasma cell neoplasms (PCNs) are clonal B-cell tumors that range from stable disease without functional abnormalities (monoclonal gammopathy, synonym monoclonal gammopathy of unknown significance) to one of slowly proliferating plasma cells (smoldering myeloma [SMM]), to one resulting in end-organ compromise (myeloma). PCNs are accompanied by the synthesis and release into the plasma of a monoclonal (M) protein and, in the case of myeloma, either diffuse osteoporosis or osteolytic lesions. Myeloma accounts for approximately 1 percent of all malignant diseases and 10 percent of hematologic malignancies.

Approximately two-thirds of patients presenting with an M protein have (1) monoclonal gammopathy (Chap. 17), whereas approximately 15 percent have (2) myeloma (Chap. 18). Other diseases associated with M-protein productions are (3) immunoglobulin light-chain amyloidosis (AL) (10 percent; Chap. 19) resulting from the deposition of immunoglobulin (Ig) fragments in visceral organs as a consequence of extensive misfolding of these Ig fragments; (4) SMM (3 percent; Chap. 18); (5) Waldenström macroglobulinemia (3 percent; Chap. 20); (6) lymphoproliferative disorders (2 percent; Chap. 1); (7) solitary or extramedullary plasmacytomas (1 percent; Chap. 18); and (8) miscellaneous other diseases (2 percent).<sup>1</sup>

### NORMAL B-CELL DEVELOPMENT

Regarding B-cell development, in brief, B-cell lymphopoiesis occurs initially in the marrow and in lymphoid tissues. In the marrow, the pro-B cell undergoes rearrangement of immunoglobulin heavy chain (IGH) genes and then is designated a pre-B cell, which is characterized by the presence of cytoplasmic  $\mu$  chains. Subsequent rearrangement of the light chain enables the cell to express surface IgM, the immature B lymphocyte phase of development. These cells leave the marrow and upon entering the blood express surface IgD, which then defines them as virgin B cells, also characterized by  $G_0$  cell-cycle arrest. Virgin B cells enter the lymphoid tissue, where they are exposed to antigen-presenting cells, become activated when in contact with the corresponding antigen, and differentiate into short-lived, low-affinity plasma cells or memory B cells. These memory B cells travel from the extrafollicular area of the lymph node to the primary follicles, where if confronted with an antigen, presented by follicular dendritic cells, a secondary response is induced. At this stage, primary follicles change into secondary follicles containing germinal centers. Through activation by an antigen, the memory B cells differentiate into centroblasts, resulting in Ig isotype switching and somatic mutations in the variable region of the immunoglobulin gene with the generation of high-affinity antibodies. Centroblasts progress to the centrocyte stage and reexpress surface Ig. The centrocytes with high-affinity antibodies differentiate into either memory B cells or

**Acronyms and Abbreviations:** AL, light-chain amyloidosis; BAFF, B-cell activating factor; BCR, B-cell receptor; BMSC, bone mesenchymal stem cell; BTK, Bruton tyrosine kinase; CDR, complementarity determining regions of the heavy chain; CR, complete remission; CSC, cancer stem cell; D, diversity immunoglobulin gene segment; FISH, fluorescence *in situ* hybridization; FLC, free light chain; GFR, glomerular filtration rate; ICAM-1, intercellular adhesion molecule 1; Ig, immunoglobulin; IGH, immunoglobulin heavy chain; IGF-1, insulin-like growth factor 1; IL, interleukin; IRAK, interleukin-1 receptor-associated kinase; JAK2/STAT3, Janus kinase 2/signal transducers and activators of transcription;  $J_H$ , joining region immunoglobulin gene segment; M, monoclonal; MBD, myeloma bone disease; MPC, multiparameter flow cytometry; MRD, minimal residual disease; MRI, magnetic resonance imaging; mSMART, Mayo Stratification of Myeloma and Risk-Adapted Therapy; MYD, myeloid differentiation primary response gene; nCR, near complete remission; NEK2, a serine/threonine kinase; NF- $\kappa$ B, nuclear factor  $\kappa$ B; OB, osteoblast; OC, osteoclast; OL, osteolytic lesion; OPG, osteoprotegerin; PCN, plasma cell neoplasm; PDGF, platelet-derived growth factor; PET/CT,  $^{18}$ F-fluorodeoxyglucose positron emission tomography-computed tomography; pP-7, a hyperphosphorylated protein; RAG, recombinase-activating genes; RANK, receptor activator of NF- $\kappa$ B; RAR $\alpha$ , retinoic receptor  $\alpha$ ; RB, retinoblastoma gene; sCR, stringent complete remission; sIFE, serum immunofixation electrophoresis; SMM, smoldering myeloma; SP, side population; SPEP, serum protein electrophoresis; TGF- $\beta$ , transforming growth factor  $\beta$ ; TLR, toll-like receptor; TME, tumor microenvironment; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; TRAF3, the adaptor molecule for toll receptor; uIFE, urine immunofixation electrophoresis; UPEP, urine protein electrophoresis; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor;  $V_H$ , the variable immunoglobulin gene segment.



plasmablasts, which then move to the marrow and terminally differentiate to plasma cells. Marrow plasma cells produce most of the plasma immunoglobulins and have a life span of approximately 3 weeks.

Three distinct gene segments, the variable ( $V_H$ ), diversity (D), and joining region ( $J_H$ ) genes, encode the variable region of the heavy chain, whereas two segments, variable ( $V_K$  or V) and joining ( $J_K$  or J) region genes, encode the variable fraction of the light chain. The IGH locus on chromosome 14q32 contains an estimated 100 to 150  $V_H$  genes, 30 D, and six  $J_H$  gene segments. Because some of the  $V_H$  genes are nearly identical, it is likely that 60 to 70  $V_H$  genes are available for rearrangement. These 60 to 70 genes belong to seven families ( $V_H$  1 to 7) whose members have more than 80 percent sequence homology. Of the 75 known  $V_K$  sequences, only 36 are potentially functional, and of the 36 known V sequences, only 24 are functional. Rearrangement of the V gene segments is dependent on the protein products of the recombinase-activating genes RAG-1 and RAG-2. Recombination of V genes starts in lymphoid progenitors within the IGH locus of either the maternal or the paternal chromosome 14. If the initial  $V_H$ -D- $J_H$  rearrangement yields a sequence that cannot be translated, then rearrangement of the IGH locus proceeds on the other allele. The presence on the B-cell surface of a fully assembled  $\mu$  heavy-chain rearrangement begins when one of the  $V_K$  genes rearranges to one of the  $J_K$  genes. If  $\kappa$  light-chain rearrangement is unsuccessful on both alleles, by default light chains will subsequently rearrange. The Ig heavy and light chains each contain three hypervariable complementarity-determining region segments, which are the areas of the immunoglobulin in direct contact with the antigen. In a process of trial and error, immunoglobulins increase their affinity for an antigen by a series of somatic mutations. Of the complementarity-determining regions (CDRs) of the IGH, CDR 3 is the most variable portion of the Ig molecule because it not only contains somatic mutations as is the case for CDR 1 and CDR 2, but also encompasses the 3' end of  $V_H$ , all of D, and the 5' end of  $J_H$ . It is, therefore, an ideal marker to detect a very small population of the malignant myeloma clone within a larger population of normal cells.

## ETIOLOGY AND PATHOGENESIS

### ETIOLOGY OF PLASMA CELL NEOPLASM

#### *Monoclonal Gammopathy*

Although monoclonal gammopathy (Chap. 17) shares the same constellation of risk factors and cytogenetic abnormalities with myeloma, it is an antecedent neoplasm that may undergo clonal evolution to any one of the PCNs or to a B-cell lymphoma.<sup>2</sup> Two studies have reported that monoclonal gammopathy is a precursor to myeloma in virtually all cases.<sup>2,3</sup>

Retrospective population-based cohort studies have established that nearly 80 percent of cases of myeloma develop from IGH monoclonal gammopathy. The remaining 20 percent have serum-free light-chain (FLC) monoclonal gammopathy. The prevalence of FLC monoclonal gammopathy is 0.8 percent in the general population. It progresses to myeloma in a minority of patients at the rate of 0.3 percent per year,<sup>1</sup> much lower than the conventional monoclonal gammopathy progression rate of approximately 1 percent.

Factors such as chronic antigen stimulation and chemical exposure have been suspected in the development of monoclonal gammopathy and other PCNs. Some studies have found a positive association,<sup>4,5</sup> but the results have not been consistent, and given our current understanding of the genetic precedents of myeloma, one would have to show a direct effect of such agents on the causal mutations involved.

A familial history of monoclonal gammopathy and myeloma has been reported to be a risk factor for developing the disease in first-degree

relatives, including a population-based study from the Mayo Clinic. A twofold increased relative risk was noted for the development of monoclonal gammopathy among the first-degree relatives of myeloma and monoclonal gammopathy patients. In a large Swedish population study, among first-degree relatives of patients with monoclonal gammopathy, a threefold increased risk for both monoclonal gammopathy and myeloma, a fourfold risk of developing Waldenström macroglobulinemia, and a twofold risk of developing B-cell chronic lymphocytic leukemia was observed.<sup>6</sup> These observations support the role of both germline susceptibility genes and possibly immune-related phenomena in the causation.<sup>7</sup> Because of the extremely low lifetime risk of developing myeloma in the general population (0.2 percent), it would be inefficient to screen the first-degree relatives of persons with myeloma or monoclonal gammopathy.

Hyperphosphorylated paratarg-7, a frequent autoantigenic target of human paraproteins, is linked to both familial and nonfamilial forms of monoclonal gammopathy and myeloma.<sup>7</sup> Paratarg-7 is the target of 15 percent of the monoclonal proteins of the IgA and IgG type and 11 percent of IgM-monoclonal gammopathy or macroglobulinemia patients. All patients with paratarg-7-specific paraproteins were carriers of a hyperphosphorylated protein (pP-7); this hyperphosphorylation is inherited in a dominant fashion. Hyperphosphorylation is a result of inactivation of phosphatase 2A.<sup>8</sup> Hyperphosphorylated paratarg-7 carriers are most prevalent among Americans of African defined single risk factor for monoclonal gammopathy or myeloma in all ethnic groups<sup>9</sup> and is associated with a sixfold increased risk of IgM monoclonal gammopathy or macroglobulinemia.<sup>10</sup>

Nearly 50 percent of patients with monoclonal gammopathy have plasma cells with translocations involving the IGH locus on chromosome 14q32 and one of the five partner chromosomes: 11q13 (cyclin D1 gene), 4q16,3 (FGFR-2 and MMSET), 6q21 (CCND3), 16q23 (c-maf), and 20q11 (maf-B).<sup>11-14</sup>

Unfortunately, none of the molecular or chromosomal abnormalities associated with myeloma predict the evolution of monoclonal gammopathy to myeloma. Two clinical risk stratification models propose high-risk features that can predict progression from monoclonal gammopathy and SMM to myeloma.<sup>15,16</sup> While one model uses the type of immunoglobulin, quantity of M protein, and the serum FLC ratio to determine the risk of progression, the other model is based on flow cytometry findings of aberrant plasma cells, marrow plasma cell percentage, DNA aneuploidy, and immune paresis (a decrease in noninvolved immunoglobulins).

#### *Smoldering Myeloma*

SMM is discussed in Chap. 18. In addition to the marrow plasma cell burden and quantitative M protein (>3 g/dL), presence of light-chain proteinuria and IgA M-heavy chain were identified as separate risk factors predicting progression to active myeloma.<sup>17-20</sup> The median time of progression to myeloma has been reported to range between 3 and 8 years in low-risk groups and between 1 and 2 years in high-risk groups.<sup>17-22</sup> Some studies have also investigated the use of magnetic resonance imaging (MRI) in detecting skeletal abnormalities not seen on a skeletal survey; time to progression to myeloma was much shorter in patients with focal lesions on the MRI,<sup>23</sup> and these patients should be treated.

A number of models estimating the risk of progression to myeloma have been proposed. The presence of serum M protein of greater than 3 g/dL, an FLC ratio outside the reference range of 0.125 to 8, and greater than 10 percent plasma cells in the marrow represents SMM with a high-risk of progression. Patients with these three risk factors had a cumulative risk of 76 percent of progression to myeloma within 5 years.<sup>15,24</sup> The risk of progression decreased to 51 percent in patients with two of the risk factors and to 25 percent in patients with a single risk factor.<sup>24,25</sup>

Another clinical risk stratification model uses flow cytometry of marrow aspirates. Using as risk factors (1) greater than 95 percent of all plasma cells being aberrant at diagnosis, (2) DNA aneuploidy, and (3) immune paresis, the presence of one, two, or three risk factors translated to 4, 46, and 72 percent risk of progression to myeloma with 5 years of observation, respectively.<sup>16</sup> Other researchers have found that, in addition to the intrinsic, molecular, and cytogenetic abnormalities of the plasma cells, an angiogenic switch and immunologic factors play a key role in the transformation of SMM to established myeloma.<sup>26</sup>

### Myeloma

The development of myeloma is a complex multistep process involving karyotypic instability, Ig translocations, cell-cycle abnormalities (cyclin Ds), and multiple other mutations<sup>27</sup> (Chap. 18). No molecular or chromosomal abnormalities can distinguish among monoclonal gammopathy, SMM, or myeloma at the time of diagnosis. Certain mutations occur in much higher frequencies in myeloma, such as p53 deletions, especially in refractory and extramedullary presentations,<sup>12,28</sup> *N-RAS* and *K-RAS* mutations, chromosome 1p deletion and gain of 1q21, and translocations involving *MYC* (8q24).<sup>29–33</sup> In whole-exome sequencing studies, intracлонаl heterogeneity has been shown to be present at all stages of development—from monoclonal gammopathy to SMM to progressive myeloma.<sup>34</sup> Genetic complexity increases as the disease progresses to myeloma.

**Effect of Endogenous Factors** There is an increased relative risk of monoclonal gammopathy and myeloma in overweight and obese patients based on their body mass index (BMI). With the exception of elite athletes, a BMI of 25.0 to 29.9 kg/m<sup>2</sup> and 30 kg/m<sup>2</sup> or greater define overweight and obese individuals, respectively.<sup>35–42</sup> Fat tissue is a dynamic endocrine organ, secreting adipokines, hormones that play an important role in energy homeostasis and inflammation. Several adipokines, such as leptin and adiponectin, have been implicated in the development of cancer.<sup>40</sup> Adiponectin levels are inversely correlated with obesity. Adiponectin serum concentrations were lower in monoclonal gammopathy patients who subsequently developed myeloma. In the KaLwRij strain of C57 black mice, which is permissive to the growth of 5T myeloma, compared to the parental strain of C57B16, which is not, adiponectin gene expression is significantly lower than in the parental strain. An increased myeloma burden was found in adiponectin-deficient mice, while pharmacologic enhancement of circulating adiponectin resulted in apoptosis of myeloma cells and also prevented bone disease. Fat tissue is a principal source of interleukin (IL) 6, one of the principal growth and antiapoptotic cytokines acting on myeloma cells. Obese individuals have been shown to have shorter telomeres than nonobese individuals. Because telomeres protect chromosomes from injury, including undesirable translocations, this effect may also contribute to the relationship of body mass with PCN.

**Effect of Exogenous Factors** Aspirin has been shown not only to reduce cancer incidence but also to dramatically decrease cancer mortality, especially in colorectal, esophageal, gastric cancer, breast, prostate, and lung cancer. Aspirin inhibits several pathways that are important in myeloma, including nuclear factor kappa B (NF-κB), AKT activation, and the BCL-2 family of proteins. Aspirin is used frequently as thromboprophylaxis in myeloma patients receiving immunomodulatory therapy. In a prospective study designed to examine whether regular aspirin use influences the risk of myeloma, participants taking five or more tablets of 325 mg per week had a 39 percent lower myeloma incidence than nonusers. The association appeared stronger in men than in women.<sup>43</sup> Aspirin inhibits proliferation and induces apoptosis of myeloma cell lines *in vitro* through regulation of BCL-2 and BAX and suppression of vascular endothelial growth factor (VEGF). In addition, *in vivo* studies in mice showed that aspirin administration resulted in

retardation of tumor growth and in increased survival.<sup>44</sup> A number of case-control and cohort studies have established that smoking has no association with the incidence of myeloma. Convincing evidence has not been found linking alcohol consumption to myeloma development.<sup>45</sup>

**Occupation** Many studies evaluated the potential role of exposure to certain occupations and/or toxins and the subsequent risk of myeloma development. Agricultural workers and farmers were studied in the United States and Europe. The majority of studies reported an increased frequency of myeloma in agricultural workers, whereas other reports failed to find such a correlation.<sup>46</sup> Exposure to toxins such as organic solvents (e.g., toluene, benzene), pesticides, paints, and other products with trace benzene content has been investigated for an association with the incidence of myeloma, but the findings are inconsistent.<sup>47</sup>

**Radiation** Studies on the survivors of the atomic bombing in Japan have failed to establish a cause-and-effect relationship between high-dose radiation exposure and an increased incidence of myeloma.<sup>48</sup> Studies from the United Kingdom have reported no increased frequency of myeloma in workers exposed to ionizing radiation, nuclear plants, and/or plutonium.<sup>45</sup> In a large study in China of x-ray technicians, there were no reported cases of myeloma or plasma cell disorders.<sup>49</sup> Thus, radiation exposure is not linked to the risk of myeloma.

Chronic immune stimulation has not been shown to play a causative role in the etiology of myeloma. No link between infections, allergic conditions, or immunizations and the development of myeloma has been established. Patients with autoimmune disorders, in general, have not been found to have an excess risk of myeloma. Some studies reported an increased risk of myeloma in HIV<sup>50,51</sup> and hepatitis C patients,<sup>52</sup> although more convincing data are needed to establish a cause-and-effect relationship.

### Waldenström Macroglobulinemia

**Etiology** In a small study of 65 patients and 213 controls, a preceding autoimmune condition did not predict the development of Waldenström macroglobulinemia.<sup>53</sup> In contrast, many other studies have found such a link. In a large population-based study, which included 146,394 hepatitis C patients and 572,293 controls, a threefold increased risk of macroglobulinemia was observed along with a 20 to 30 percent increased risk of lymphoma.<sup>54</sup> In a study of 361 U.S. veterans with Waldenström macroglobulinemia after a 27-year followup, a two- to threefold increased risk of developing the disease was found in patients with autoimmune-related conditions, hepatitis, HIV infection, and rickettsiosis.<sup>55</sup> In two Swedish population-based studies, a personal history of autoimmune conditions and infections was associated with an increased risk of macroglobulinemia.<sup>56</sup>

Case-control studies and a large population-based study in Sweden have established the role of familial clustering of macroglobulinemia, thereby raising the concept of common susceptibility genes that could predispose to the disease.<sup>53,57–61</sup> In a study of macroglobulinemia, 19 percent of patients had at least one identified first-degree relative with macroglobulinemia or a B-cell disorder.<sup>60</sup> In genome-wide linkage analyses of high-risk families with macroglobulinemia and IgM monoclonal gammopathy, the strongest linkage was found to be chromosomes 1q, 3q, 4q, and 6q.<sup>58,60,62–64</sup> In a gene-sequencing study performed on marrow cells of macroglobulinemia patients, a recurrent somatic mutation of the gene *MYD88L265P* on chromosome 3 that encodes signal transduction and innate immunity was found to be in approximately 91 percent of the patients tested.<sup>60</sup>

In a novel study using expression cloning, several common self-antigens designated paratarg-7 were discovered.<sup>10</sup> When carriers of the phosphorylated forms of paratarg-7 were studied, they were found to have a 6.5-fold higher risk of developing IgM monoclonal gammopathy or macroglobulinemia. The antigen causes continuous autostimulation of cognate T-helper cells, which in turn specifically activate B cells with high affinity to paratarg-7.

## ● GENETIC ABNORMALITIES IN PLASMA CELL NEOPLASM

### MYELOMA

Plasma cell differentiation begins in lymph nodes and in the spleen, where cells undergo changes in gene expression and cell-surface molecules followed by migration to the marrow or mucosal lamina proper.<sup>65</sup> The development of plasma cells alters the cellular receptor landscape of the cell with the deletion of important B-cell receptors (BCRs) and the addition of receptors necessary for plasma cell function and antibody production. Changes to cellular receptors include downregulation of major histocompatibility complex (MHC) class II, CD19, CD21, and CD22.<sup>66</sup> Perhaps the most important alterations during myeloma development are decreases of the B-cell receptor (BCR), CXCR5, and CCR7. In contrast, plasma cells upregulate CXCR4, CD138, and CD38 (Fig. 16-1).<sup>66-68</sup> Plasma cells also undergo changes to transcription factors highlighted by a decrease in PAX5, CIITA, and EBF.<sup>66-70</sup> Furthermore, plasma cells express genes that are present in B cells at low levels or not at all and are highlighted by increased expression of Blimp-1, IRF4, and XBP-1, the only transcription factor exclusively required for plasma cell development.<sup>71</sup>

#### Early Genetic Events in Myeloma Genesis

Early genetic events in myeloma genesis include the accumulation of sequential genetic changes; however, the full mechanism remains elusive.<sup>72</sup> Protein kinases provide selective growth advantages to cells and therefore act as driver mutations, or inducers of early neoplastic events.<sup>73</sup> The dysregulation of the cyclin D genes exposes cells to additional proliferative stimuli and commonly occurs as a result of a translocation of the cyclin D gene to the Ig loci.<sup>74</sup> Activation-induced deaminase contributes to genetic instability through its involvement in induction of somatic mutations in the immunoglobulin genes and immunoglobulin translocations.<sup>75</sup> Although important, gene alterations are not the only driving force in the development of PCN. Although ethnicity and genealogy affect the prevalence of monoclonal gammopathy,<sup>76</sup> they do not impact the rate of progression from monoclonal gammopathy to myeloma. Furthermore, there is a higher incidence in monoclonal gammopathy in relatives of myeloma patients than seen in the general public.<sup>77,78</sup> These results demonstrate that not only are there

somatic mutations important to the development of PCNs, but also genetic background contributes to the likelihood of the development of plasma cell diseases.

#### Late Events in Myeloma Progression

Much focus has been placed on risk factors and early genetic events that can be used as diagnostic and prognostic markers. However, we are learning more about the genetic alterations that occur throughout disease progression. One of the more prominent discoveries focuses on the activation of NF- $\kappa$ B. Approximately 50 percent of myeloma patients exhibit canonical NF- $\kappa$ B activation.<sup>79,80</sup> NF- $\kappa$ B functions, in part, by regulating growth and survival within the myeloma cells; overexpression of positive regulators, such as NIK, NFKB1, NFKB2, and CD40, and inactivation of negative regulators, such as CLYD, TRAF2, TRAF3, and cIAP1/cIAP2, contribute to the constitutive activation of NF- $\kappa$ B within these cells.<sup>80</sup>

RAS mutations are also important contributors to the development of myeloma from monoclonal gammopathy. Oncogenic activation of RAS occurs through mutation of one of three different codons, with mutations resulting in constitutively activated RAS. Less than 5 percent of cases of monoclonal gammopathy display mutations of RAS, whereas in newly diagnosed myeloma patients mutated RAS is found in nearly 40 percent of patient samples, suggesting that RAS may be associated with the conversion of monoclonal gammopathy to myeloma.<sup>81-83</sup>

*p53*, which regulates the cell cycle and acts as a tumor suppressor, represents the most commonly inactivated tumor-suppressor gene in cancer.<sup>84,85</sup> In newly diagnosed PCNs, *p53* mutations occur in 5 percent of patients. The frequency of *p53* mutations increases with disease progression; while infrequent in newly diagnosed myelomas, 30 percent of plasma cell leukemia patients present with *p53* mutations.<sup>86,87</sup> Furthermore, *p53* mutations are negatively correlated with survival.<sup>88</sup> Similar in function to *p53*, the retinoblastoma (*RB*) gene regulates the cell cycle. *RB* functions by inhibiting the effects of the cyclin D proteins with the help of the *p18<sup>INK4</sup>* proteins. However, overexpression of cyclin D or decreased expression of *RB* can lead to cell-cycle progression and neoplastic growth. Decreased expression of the two *RB* pathway regulators *p18<sup>INK4a</sup>* and *p18<sup>INK4c</sup>* can inhibit the regulatory effects of *RB* and result in cell-cycle release. Decreased expression of the two proteins is considered to be a late disease progression event.<sup>76</sup>

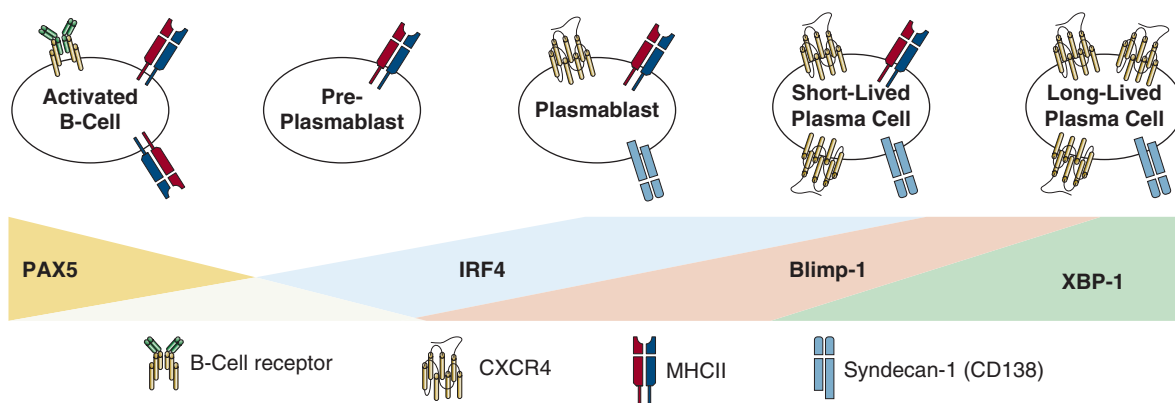


**Figure 16-1** Overview of plasma cell differentiation. The differentiation of a plasma cell from a B-cell lineage occurs over a multistep process using plasmablast and short-lived plasma cell intermediates. Throughout the process, there is a profound change in gene expression and cell-surface markers, which allows for the identification of each stage based on the cell-surface marker expression and gene expression of transcription factors important to plasma cell differentiation. Depicted here are necessary changes to transcription factors and cell-surface markers that occur during each phase of plasma cell differentiation. MHCII, major histocompatibility complex class II.



### Genetic Expression Changes in Myeloma

Myeloma is genetically heterogeneous and more closely resembles solid tumors than other hematologic malignancies. Because of its high degree of tumor heterogeneity, myeloma gene-expression microarrays have proven invaluable to our understanding and treatment of myeloma. Four stratification models exist designed to classify myeloma based on the risk profile as determined by gene-expression profiles (GEPs).<sup>89</sup> Of the four, one model has proven most reliable, as it retains its prognostic relevance with newer therapeutic regimens. This model compares the expression of 70 different genes to devise a scoring system that ranks a sample as high risk (13 percent of patients) or low risk.<sup>90</sup> Abnormalities of expression that map to chromosome 1 are disproportionately represented within the model, with an impressive number of upregulated genes mapping to 1q and a high percentage of downregulated genes mapping to 1p. The 70-gene model offers a reliable method for the detection of high-risk myeloma. However, it does not take into account all important genes related to myeloma. One important gene not found in this model is *MYC*, which is a transcription factor that influences the expression of many genes through the binding of consensus sequences within the noncoding region of genes. It is thought to regulate the expression of 15 percent of all genes.<sup>91</sup> *MYC* expression within myeloma and monoclonal gammopathy varies depending on the state of the disease. In high-risk monoclonal gammopathy and early stages of myeloma, the increase in *MYC* expression is primarily a result of a decrease in transcriptional regulation, whereas in late disease states, the 8q region encoding *MYC* translocates to the immunoglobulin loci, resulting in dysregulation of expression because of the influence of neighboring regulatory elements.<sup>32</sup>

### Drug Resistance

Refractory myeloma has several causes, with dysregulation of gene expression contributing significantly to the overall development of drug resistance. The serine/threonine kinase *NEK2* induces drug resistance in myeloma through the activation of efflux drug pumps.<sup>92</sup> Overexpression of *NEK2* results in the activation of the *AKT* pathway and subsequently of *NF-κB*, which results in the upregulation of ABC drug efflux transporters. Overexpression of the antiapoptotic molecule *MCL-1* induces drug resistance through the inhibition of apoptosis.<sup>93</sup> More specifically, *MCL-1* overexpression results in the inhibition of the proapoptotic *BCL-2* family members, which results in blocking apoptosis.

### Next-Generation Sequencing

Next-generation sequencing or deep sequencing relies on the ability to sequence large amounts of small DNA fragments quickly and assemble the output into a complete coherent data set. This technique is providing new insights into our understanding of existing neoplastic mechanisms and identifying novel mechanisms and genes that had evaded detection thus far. One of the first reports to use next-generation sequencing was from a study that compared 38 whole-tumor genomes from patients with myeloma to matched normal DNA.<sup>94</sup> Novel genes involved in histone methylation, protein translation, and blood coagulation were identified. Mutations to the toll-like receptor (TLR) 4, to the adaptor molecule *TRAF3*, and to the kinases *CYLD*, *RIPK4*, and *BTRC* also were discovered. A greater-than-anticipated change in *NF-κB* was observed, including identifying 11 mutated genes involved in the *NF-κB* pathway. Furthermore, the prognostic value of next-generation sequencing was tested by using the sequencing technology to detect minimal residual disease (MRD) in myeloma patient samples.<sup>95</sup>

### Cytogenetic Microarrays

Fluorescence *in situ* hybridization (FISH) has become the gold standard for the detection and classification of myeloma. However, FISH cannot provide information about chromosomal abnormalities without

the use of large-scale panels of probes. Comparative genomic hybridization (CGH) arrays overcome some of the shortfalls of FISH technology by providing a genome-wide view of chromosomal changes, but do so at a reduced resolution of 10 to 20 megabases. To combat the low resolution of CGH arrays, technology based on small nucleotide polymorphisms (SNPs) has been employed. SNP arrays can detect copy number changes and have improved the resolution of CGH arrays to a submegabase level. An early report validated the use of SNP arrays by comparing its results to those obtained by FISH analysis using identical samples. Furthermore, uniparental disomy (UPD) was identified as prevalent in myeloma samples and may occur through several mechanisms, including mitotic nondisjunction and mitotic recombination.<sup>96</sup> A technical advance was the introduction of the Affymetrix Cyto Scan HD arrays. The Cyto Scan arrays incorporate the most up-to-date SNP library to generate a library of 2.6 million probes across the human genome, allowing for resolution up to 50 kb. The technology has not been published in a myeloma study yet, but the resolution matches that of FISH and can provide a wealth of knowledge related to changes in copy number, mosaic chromosomes, and loss of heterozygosity. Using these technologies, numerous cytogenetic changes were identified that may be important for the development and progression of monoclonal gammopathy to myeloma.

## TECHNIQUES TO ASSESS CYTOGENETIC INFORMATION

### FLUORESCENCE IN SITU HYBRIDIZATION

In PCNs, FISH has emerged as one of the most useful and reliable methods for the detection of chromosomal abnormalities and performing risk assessment on newly diagnosed patients. G-banding cytogenetic analysis requires actively dividing cells for identification of chromosomal changes, not commonly seen in plasma cells, whereas FISH analysis can overcome this limitation to an extent and detect structural abnormalities in nondividing plasma cells.<sup>97</sup> A panel of FISH probes is used to query for commonly identified cytogenetic changes, including t(4;14), t(11;14), t(14;16), t(14;20), t(6;14), del(13q14), and del(17p13) and probes for hyperdiploidy (5, 7, 9, 11, 15, and 17). Cytogenetic abnormalities, using FISH, are observed in more than 90 percent of myeloma patients. Panels like the one described here provide essential information and paired with GEPs provide clinicians with essential data for diagnosis and the development of treatment regimens.

### METAPHASE CYTOGENETICS

Approximately 30 percent of patients with newly diagnosed myeloma present with chromosomal abnormalities by metaphase cytogenetics, and this percentage increases to 50 percent in patients with relapsed myeloma. Cytogenetic changes broadly divide patients into one of two categories: patients with hyperdiploid chromosomal profile and those with nonhyperdiploid profiles, encompassing hypodiploid and hypotetraploid cases. Hyperdiploid cytogenetic profiles are characterized by trisomies of many odd-numbered chromosomes, namely, 3, 5, 7, 9, 15, 19, and 21, and are associated with a favorable outcome.<sup>98</sup> Nonhyperdiploid profiles are highly enriched with translocations at the IGH loci (14q32) with partner chromosomes, most importantly resulting in t(4;14), t(6;14), t(11;14), t(14;16), and t(14;20).<sup>97</sup> However, many of the translocations result in the activation of cell-cycle regulators and oncogenes like cyclin D, *MMSET* and *c-MAF*, *MAFB*. In addition, amplification of chromosome 1q and deletions to chromosomes 13q and 17p are commonly seen in myeloma patients.<sup>96</sup> With regard to deletion of chromosome 17p, the number of patients presenting with this abnormality

increases as the disease progresses.<sup>85,96</sup> To accommodate the variability in cytogenetic markers, researchers have developed diagnostic criteria to aid in the management of patients with a variety of cytogenetic abnormalities with prognostic value and are discussed next.

## CYTOGENETIC ABNORMALITIES AS PROGNOSTIC MARKERS IN PLASMA CELL NEOPLASM

One model has become the standard for myeloma prognostic risk assessment: the Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART).<sup>99,100</sup> Historically, risk assessment was categorized into two groups, standard or high risk. The mSMART guidelines add an intermediate-risk subgroup to better assess the appropriate treatment regimen. The standard-risk group consists of patients presenting with either the t(6;14) or the t(11;14) translocation as well as the hyperdiploid group, whereas the intermediate-risk group comprises patients presenting with the t(4;14) translocation and deletions of chromosome 13 or hypodiploidy; the high-risk group is made up of patients presenting with the t(14;16) or the t(14;20) translocation or deletion 17p13. Patients with deletion of chromosome 13 by metaphase cytogenetics (not by FISH) are also considered high risk. The mSMART guidelines do not take into account all cytogenetic abnormalities seen in myeloma samples. There has been considerable research performed to identify prognostic markers within patients, including mRNAs and cell-surface receptors such as CD20+ samples as a prognostic marker.<sup>101</sup> Chromosome 1 changes are important markers in myeloma; patients harboring the 1p deletion have a poor outcome.<sup>102</sup> The amplification of chromosome 1q has also been identified as an important locus in myeloma. Amplification of 1q21 is a poor prognosis marker in PCN and the frequency of 1q21 amplifications increases as the disease progresses.<sup>30</sup> Furthermore, studies have identified the chromosome 1q genes *NEK2* and *CKS1B* as markers of aggressive disease and poor prognosis.<sup>92,103</sup> Presently, alterations to chromosome 1 are the only chromosomal markers not incorporated into the commonly accepted prognostic models.

## GENETIC ABNORMALITIES IN IMMUNOGLOBULIN LIGHT-CHAIN AMYLOIDOSIS

The clonal plasma cell burden in AL is usually small and similar to that seen in patients with monoclonal gammopathy. Because the proliferation rate of plasma cells is very low, chromosomal aberrations need to be assessed by FISH analysis and not by conventional metaphase cytogenetics. Approximately 70 percent of patients with AL have FISH abnormalities, the most common being IgH translocations (48 percent), including t(11;14) and t(14;16). Other chromosomal abnormalities seen in AL include deletion 13/13q- and hyperdiploidy.<sup>104</sup> The t(11;14) occurs more frequently in AL (39 percent) than in monoclonal gammopathy or myeloma.<sup>105</sup> Although cases with t(4;14) abnormality have been reported, those are exceptional, and deletion 17p13 is not seen in this form of amyloidosis. FISH analysis is important in this disease because t(11;14) is associated with an inferior prognosis in amyloidosis in contrast to myeloma, where it is associated with a good prognosis. In another large study assessing the prognostic significance of cytogenetic abnormalities, t(11;14) was not associated with an inferior outcome, but gain of chromosome 1q21 clearly was.<sup>106</sup>

## GENETIC ABNORMALITIES IN WALDENSTRÖM MACROGLOBULINEMIA

Alterations in one of the myeloid differentiation primary response genes, *MYD88*, have been identified in 90 percent of patients with

macroglobulinemia.<sup>107</sup> *MYD88* is an adaptor protein important in TLR and IL-1 receptor (IL-1R) signaling pathways. *MYD88* is recruited to the activated receptor complex as a homodimer that complexes with the IL-1R-associated kinase (IRAK) 4 to activate IRAK 1. It leads to NF- $\kappa$ B activation via I $\kappa$ B  $\alpha$  phosphorylation. Cell survival is enhanced by *MYD88* overexpression, which leads to Bruton tyrosine kinase (BTK) phosphorylation. Combined use of IRAK and BTK inhibitors results in synergistic killing of *MYD88*-expressing macroglobulinemia cells. High rates of response have been observed in a clinical trial with a BTK inhibitor in relapsed and refractory patients.<sup>108</sup>

## THE MARROW MICROENVIRONMENT

The marrow microenvironment provides a highly supportive tumor microenvironment (TME) for the development, growth, and survival of neoplastic cells in patients with myeloma. In the great majority of cases, the clonal expansion of these cells in the marrow is associated with increased blood vessel formation (neoangiogenesis) and, more importantly, myeloma bone disease (MBD).

Nonmalignant stromal cells in the marrow secrete cytokines and chemokines that promote myeloma cell growth and survival upon binding to specific receptors on the myeloma cell surface. Tumor promoters include IL-6, insulin-like growth factor 1 (IGF-1), VEGF, B-cell activating factor (BAFF), fibroblast growth factors (FGFs), stroma cell-derived factor 1 $\alpha$  (SDF-1 $\alpha$ , also known as C-X-C motif chemokine 12 or CXCL12), and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ). Direct physical interaction of myeloma and marrow stromal cells by virtue of cell-to-cell adhesion may further enhance the cellular signaling pathways that are activated by cytokines and chemokines, thereby facilitating migration of myeloma cells to distant marrow and/or extramedullary sites (tumor dissemination). Myeloma-to-bone mesenchymal stem cell adhesion is also involved in the acquisition of drug resistance by tumor cells, underlying tumor relapse and/or refractory disease in patients with myeloma.<sup>109</sup>

## HOMING AND ADHESION OF MYELOMA CELLS

Homing and adhesion of myeloma cells to the marrow microenvironment involves the CXCL12/CXCR4 pathway and a number of homo- or heterotypic adhesion factors, including CD44 (an anionic, nonsulfated glycosaminoglycan called hyaluronan), very-late antigen 4 (VLA-4, composed of integrins  $\alpha_4$  [CD49d] and  $\beta_1$  [CD29]) and its receptor, vascular cell adhesion molecule 1 (VCAM-1, CD106), leukocyte function-associated antigen 1 (LFA-1, CD11a), neuronal cell-adhesion molecule (NCAM, CD56), intercellular adhesion molecule 1 (ICAM-1, CD54), and, importantly, syndecan 1 (CD138). Syndecan 1 is a transmembrane heparan sulfate-containing proteoglycan that is usually expressed at high levels on the myeloma cell surface. Syndecan 1-mediated adhesion of myeloma cells promotes adhesion-dependent drug resistance and bone resorption via expression of matrix metalloproteinases, among other mechanisms.

Adhesion of myeloma cells to mesenchymal cells activates pleiotropic cellular signal transduction pathways that mediate the proliferative and survival-enhancing response of myeloma cells upon interaction with the marrow microenvironment. Resistance of myeloma cells to cytotoxic drugs is also promoted. These pathways include NF- $\kappa$ B (nuclear factor kappa light-chain enhancer of activated B cells), PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase B/AKT oncogene), RAS/RAF/MEK/ERK (rat sarcoma protein subfamily of small GTPase/RAF protooncogene serine and threonine protein kinase/MAPK kinase/extracellular signal-regulated kinase), and JAK2/STAT3 (Janus kinase 2/signal transducers and activators of transcription 3). Engagement of these pathways results in upregulation of proteins that control cell cycle progression (e.g., D cyclins and Myc) and those that

protect myeloma cells from programmed apoptotic cell death (e.g., BCL2, BCL-x<sub>L</sub>, and MCL1).

The interplay of myeloma and mesenchymal cells is not the same in all subtypes of the disease. Instead, the genetic makeup of myeloma determines, in part, the interaction of tumor with the nonmalignant stromal cells in the marrow environment. For instance, myeloma cells harboring an MAF (v-MAF oncogene homologue)-activating chromosomal translocation t(14;16) overexpress the adhesion factor integrin  $\beta_7$ . This results in enhanced tumor cell adhesion to bone mesenchymal stem cells (BMSCs) relative to myeloma cells not containing a t(14;16).<sup>110</sup> Signaling pathways that govern interactions of myeloma and normal marrow cells also promote neoangiogenesis in the TME. The formation of new blood vessels, which is driven to a large extent by CXCL12/CXCR4 signaling and production of VEGF, B-FGFs, matrix metalloproteinases (MMPs), IGF-1, interleukins (e.g., IL-8, IL-1), angiopoietin 1, transforming growth factor  $\beta$  (TGF- $\beta$ ), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF), is critical for myeloma progression and thus an important target of myeloma drug development.

## ● MYELOMA BONE DISEASE

Increased bone resorption and suppressed bone formation lead to osteolytic lesions in patients with myeloma.<sup>111,112</sup> The main cytokines involved in that process are IL-6, receptor activator of NF- $\kappa$ B ligand (RANKL)/osteoprotegerin (OPG), BAFF, chemokine (C-C motif) ligand 3 (CCL3)/macrophage inflammatory protein (MIP)-1 $\alpha$ , and VEGF. The main cell adhesion and integrin signaling pathways involved in that process are VLA-4/VCAM-1 and LFA-1/ICAM-1.<sup>112,113</sup> WNT/ $\beta$ -catenin signaling antagonists that inhibit osteoblast differentiation in myeloma also play an important role in the natural history of MBD. These include Dickkopf 1 (DKK1),<sup>114</sup> soluble-frizzled receptor-like proteins (sFRPs), and sclerostin. Inhibition of osteoblast differentiation drives the bone disease and, presumably, facilitates the expansion of myeloma cells<sup>115</sup> because upregulation of  $\beta$ -catenin-dependent osteoblast activity *in vivo* results in significant inhibition of myeloma cell growth,<sup>116</sup> relying on an ill-defined mechanism that appears to involve the small leucine-rich proteoglycan, decorin.<sup>117</sup>

## RANK/RANKL/OPG PATHWAY

The receptor activator of NF- $\kappa$ B (RANK)-RANKL-OPG pathway is arguably one of the most important regulatory systems for homeostatic bone remodeling. RANK, a membrane-anchored signaling receptor of the TNF receptor family, is expressed on osteoclast (OC) precursors. Binding of its ligand, RANKL, expressed by osteoblasts (OBs) and BMSCs, induces osteoclastogenesis and activation of mature OCs. OPG, a soluble member of the TNF receptor family, is also produced by OBs and BMSCs. Because OPG acts as a soluble decoy receptor for RANKL, it blocks RANK-dependent OC maturation and activation. Thus, it is easy to understand that the ratio of RANKL and OPG significantly impacts the balance of bone resorption and bone deposition. Indeed, compared to normal individuals, marrow plasma of myeloma patients contains elevated levels of RANKL and reduced levels of OPG,<sup>117</sup> consistent with a proosteoclastogenic TME. Also, low serum levels of OPG correlate with advanced MBD in patients with myeloma.<sup>118</sup> In mouse models of MBD, treatment with OPG and OPG-like compounds prevented both bone destruction and myeloma growth *in vivo*.<sup>119,120</sup> Similarly, in patients with myeloma treated with thalidomide<sup>121</sup> or autologous stem cell transplantation (ASCT)<sup>122</sup> responding to therapy, the RANKL-to-OPG ratio normalized, and bone resorption was inhibited. In aggregate, these findings indicate the RANK-RANKL-OPG axis is an important therapeutic target for MBD.

## B-CELL ACTIVATION FACTOR

BAFF is a myeloma cell-promoting member of the TNF superfamily of proteins that is expressed by OCs and BMSCs. BAFF is significantly increased in the serum of patients with myeloma. In a preclinical study using a mouse model of myeloma, targeting BAFF by means of a neutralizing antibody led to reductions in tumor burden and osteolytic bone disease and increased survival.

DKK1 is an inhibitor of the WNT signaling pathway that plays a major role in MBD.<sup>114</sup> DKK1 inhibits OB differentiation and promotes OC maturation.<sup>123</sup> Patients responsive to myeloma drugs exhibit reduced serum levels of DKK1.

Activin A, a member of the TGF- $\beta$  superfamily of proteins, activates a signaling pathway that results in phosphorylation of SMADs (homologues of *Drosophila*'s mothers against decapentaplegic [MAD] and *Caenorhabditis elegans*' small body size [SMA] proteins) followed by execution of a complex SMAD-dependent gene-expression program. Activin A is a stimulator of osteoclastogenesis and is overexpressed in myeloma.<sup>124</sup> IL-3, another stimulator of OCs, can induce the secretion of activin A by resident macrophages in the myeloma marrow microenvironment, providing a mechanism for the upregulation of activin A in patients with myeloma.<sup>112</sup> Levels of activin A in the marrow plasma of myeloma patients with osteolytic lesions (OLs) are increased.<sup>125</sup> RAP-011 is a soluble mouse activin A receptor that was shown to inhibit myeloma-like tumors and prevent OLs in laboratory mice.<sup>124,126</sup> Sotatercept (ACE-011), the human version of mouse RAP-011, is a soluble receptor of activin A and is undergoing clinical trials in patients with myeloma.

## BRUTON TYROSINE KINASE

The metalloprotein BTK is a member of the Scr-related Tec family of protein kinases. BTK is a regulator of OC differentiation that is expressed in OCs (but not in OBs) and has been implicated in bone resorption. BTK is expressed in myeloma cells.<sup>127,128</sup> Genetic evidence indicates BTK may be crucial for neoplastic plasma cell development in laboratory mice.<sup>129</sup>

## ● MYELOMA STEM CELL

Major progress has been made in the treatment of newly diagnosed myeloma patients, with 80 percent achieving either a complete remission (CR) or near CR (nCR) and more than 50 percent surviving 10 years.<sup>130</sup> However, many patients ultimately relapse. GEPs remain abnormal in myeloma patients with long-lasting remission (>10 years).<sup>131</sup> This finding suggests that the persistence of a myeloma cell population with low proliferative capacity and limited sensitivity to our most intensive therapies points to the concept of a myeloma stem cell problem. There is no agreement on the specific phenotype of the myeloma stem cell, but side-population (SP) cells, which constitute a minor fraction of the myeloma cells, have the features of stem cells. Such cells have greater clonogenic potential and drug resistance, whether isolated from myeloma cell lines or primary myeloma samples.<sup>132,133</sup> Many different groups have tried to identify the phenotype of myeloma stem cells. A drug-resistant subpopulation of memory B-cell-like cells with the CD138-/CD19+/CD27+ phenotype was identified and termed *myeloma stem cells*.<sup>134,135</sup> The CD138- cell population derived from myeloma cell lines contains significantly higher levels of ALDH, a marker for stem cells. In contrast to the CD138+ cells, the CD138- cells were not affected by the drugs we typically use in myeloma, such as lenalidomide, bortezomib, dexamethasone, and cyclophosphamide.<sup>134</sup> In an earlier study, human CD34+/CD45<sub>low</sub> clonotypic myeloma cells were shown to cause lytic bone lesions in a xenograft mouse model, and these CD34+ progenitors included approximately one-third of DNA aneuploid cells.<sup>135,136</sup> Mature CD138+ myeloma cell dedifferentiation to myeloma stem cells with a



CD34+/CD138+/B7-/H1+ phenotype has been reported,<sup>137</sup> showing that myeloma cells have the plasticity to replenish the stem cell compartment if required, and that we should not think of stem cells as a static, but as a dynamic concept. CD38+/CD45- plasma cells proliferated successfully within an engrafted human fetal bone using the severe combined immune deficiency (SCID)-hu mouse model.<sup>138,139</sup> This could have been because of dedifferentiation of these more mature myeloma cells. It also has been demonstrated that the SP cells from different myeloma cell lines are able to generate more colonies than mature plasma cells. However, this SP cell population lacked correlation with CD138 expression.<sup>132,133</sup> To isolate multiple myeloma stem cells (MMSCs) from primary samples, we have relied on a functional characteristic of cancer stem cells (CSCs), the ability to pump out the Hoechst dye, rather than on membrane markers, which are more likely to change with changing environmental conditions. To this functional marker, we have added a myeloma-specific marker, either surface  $\kappa$  or  $\lambda$  light chain, based on the specific M protein. Because drug-resistant myeloma cells are enriched in patients relapsing early after tandem autologous transplants, we compared GEPs at baseline and at relapse in 51 patients. The gene most differentially expressed at relapse was the retinoic receptor  $\alpha$  (RAR $\alpha$ ). This receptor has two splice variants:  $\alpha_1$  and  $\alpha_2$ . RAR $\alpha_1$  is present on all myeloma cells; RAR $\alpha_2$  expression is only present in one-third of newly diagnosed patients, and these patients have a significantly inferior survival after tandem autotransplants.<sup>140</sup> In a subsequent study, we were able to show that increased RAR $\alpha_2$  expression conferred stem cell features to myeloma cells, such as increased drug resistance, increased clonogenic potential, activation of pathways typically found in a CSC, such as the Wnt and Hedgehog pathways, increased SP and ALDH levels and increased expression of embryonic stem cell genes, such as Nanog, Oct4, and Sox2. These same characteristics were found in the SP+ and surface light-chain-restricted cells of primary myeloma samples. We also found that RAR $\alpha_2$  expressing CD138+ myeloma cells have a much higher expression of Oct4, Sox2, Nanog, TCF1, CCND1, and ABCC3. This indicates that RAR $\alpha_2$ -positive myeloma cells show stem cell features and, therefore, have a worse outcome.<sup>141</sup>

## ● DIAGNOSIS OF PLASMA CELL NEOPLASM

### TESTS OF SERUM

Because PCNs are B-cell malignancies derived from antibody-producing plasma cells in the marrow, which continue to secrete in the vast majority, an M-protein will be found in serum and/or urine in the majority of patients. The monoclonal protein is either an intact immunoglobulin or a component of the immunoglobulin. The most common types in myeloma are IgG and IgA; in Waldenström macroglobulinemia, it is IgM. The most common screening test to identify a serum M protein is the serum protein electrophoresis (SPEP). While the quantity of M protein is often considered a marker of tumor load, myeloma cells are ineffective producers of immunoglobulins and produce 10 to 100 times less immunoglobulin per cell per day than a normal plasma cell. In addition, the more immature and more proliferative myeloma cells are, the less immunoglobulin per cell per day they secrete. In myeloma, there is an imbalance of heavy chains and light chains in favor of the light chains. In general, the more aggressive the myeloma, the more pronounced the imbalance in favor of excess light-chain secretion. The excess light chains cannot bind to heavy chains and can pass freely through the renal glomeruli. Monomeric FLCs, characteristic  $\kappa$ , are cleared from the serum in 2 to 4 hours at 40 percent of the glomerular filtration rate (GFR), while dimeric FLCs typically are cleared in

3 to 6 hours at 20 percent of GFR. Removal may take 2 to 3 days in patients with complete renal failure. This is in contrast to IgG, which has a plasma half-life of 21 days.

Although it has long been possible to measure the total amount (free plus bound to heavy chains) of light chains in the urine, our ability to measure serum FLCs became a reality early in the 21st century. This test is not only of high affinity and allows measurement of low concentrations of FLC, but it is also of high specificity and only measures unbound light chains. Serum FLCs are several orders of magnitude lower than serum light chains bound to intact immunoglobulin, and the test is based on an antibody that exclusively binds to an epitope of the FLC that is hidden if the light-chain molecule is bound to a heavy chain. Because the half-life of serum FLCs is much shorter than that of the intact immunoglobulin, serial serum FLCs allow assessment of success of therapy much earlier than serial M-protein levels. Patients with the highest amount of light chains have the worst outcome.<sup>142</sup> The serum FLC ratio is an independent prognostic factor for progression of myeloma in monoclonal gammopathy and SMM.<sup>143</sup> Incorporation of serum FLCs into a staging system also improves risk stratification for patients with AL<sup>144</sup> and levels of serum FLCs correlate with tumor burden in macroglobulinemia.<sup>145</sup> The serum FLC assay is also an early indicator of relapse in myeloma. As explained previously, both serum  $\kappa$  and  $\lambda$  concentrations increase with deteriorating renal function, but the ratio only changes slightly; with increasing renal failure the  $\kappa$ : $\lambda$  ratio gradually increases. Diseases associated with generalized increased B-cell activation frequently have high concentrations of polyclonal immunoglobulins and high polyclonal FLC, but the  $\kappa$ : $\lambda$  ratios will remain within normal range.

In addition to a SPEP and serum FLCs, it is important to perform serum immunofixation electrophoresis (sIFE) to identify the nature of the M protein and to measure the quantitative immunoglobulins. Many of the IgA and some of the IgG myelomas have their M spike in the  $\beta$ -globulin region, where the M-protein level is less reliable because there is comigration of many other proteins. If an M protein is identified on SPEP and sIFE does not identify the heavy chain, quantitative analysis of IgD and IgE should be performed. Most IgD myelomas are associated with lambda light chains.

### TESTS OF URINE

A typical myeloma workup will have a 24-hour urine collection for total protein, urine protein electrophoresis (UPEP), urine M protein, and urine immunofixation electrophoresis (uIFE). In contrast to the serum FLCs, the role of urine FLCs is much less clear and is generally not used. In the era of serum FLC assays, some experts believe that urine analysis can be eliminated. In a study by the Mayo Clinic of 428 patients, there were only two cases where a urinary monoclonal protein was missed by omitting urine analysis (0.5 percent). These two cases did not require any medical intervention.<sup>146</sup> When patients have a large amount of albuminuria in the presence of PCN with or without urine M protein, the possibility of AL should be entertained, either primary AL or AL in the context of myeloma.

### MARROW EXAMINATION

Marrow aspirate and biopsy are essential in the workup of PCN. It is best not to rely solely on a marrow aspirate. Myeloma cells have the tendency to form small or large clusters, and myeloma is not evenly distributed throughout the marrow. The marrow may be falsely negative in the presence of overt myeloma. In a normal marrow with normal cellularity, there may be up to 2 percent plasma cells present. However, the percentage of plasma cells can be much higher in reactive marrows and hypoplastic or aplastic marrows in the absence of PCN. In a normal

marrow, there can be some clustering of plasma cells around the blood vessels. However, the presence of larger clusters of plasma cells away from the blood vessels should alert one to the possibility of PCN, especially myeloma. In addition to the morphologic examination, the biopsy slides should be stained with a CD138 monoclonal antibody, which is a specific immune marker for plasma cells. If the diagnosis of PCN is not clear based on morphology and CD138 staining, *in situ* hybridization for cytoplasmic  $\kappa$  and  $\lambda$  should be used to evaluate whether the plasma cells are clonal (light-chain restricted).

It is customary to perform flow cytometry, metaphase cytogenetics, and FISH analysis on myeloma samples. Flow cytometry at diagnosis is mainly performed to identify aberrant surface markers on myeloma cells, which can be used subsequently to assess MRD. The identification of an accurate gating strategy is a critical component of a reproducible and sensitive immunophenotypic analysis. The best gating strategy uses a combination of CD38, CD138, CD45, and light-scatter characteristics. The previously used gating method of CD38 and CD45 decreases the risk of contamination with other cells, but excludes the CD45+ cells, which can constitute the majority of the plasma cells. It is recommended that at least a four-color instrument be used, as at least two antigens (CD38 and CD138) are required after the initial analysis with CD138, CD38, CD45, and light scatter to gate plasma cells accurately. When used for MRD, at least 100 neoplastic plasma cell events should be acquired. CD56 and CD19 should always be assessed. CD56 is negative on normal plasma cells but is positive on myeloma cells, while the opposite is true for CD19. It is also recommended to stain for CD117 and CD20, which are negative on normal plasma cells and can be aberrantly expressed on myeloma cells. CD28 and CD200 are negative or only weakly expressed on normal plasma cells but can be strongly positive on myeloma cells, whereas CD27 and CD81 are strongly expressed on normal plasma cells, but weak or negative on myeloma cells. It is important to remember that there is no single marker that can systematically differentiate neoplastic cells from normal plasma cells. Also, the percentage of plasma cells detected by flow cytometry is typically lower than that found by morphology.<sup>147</sup> The importance of metaphase cytogenetics and FISH analysis was outlined in the techniques section to assess cytogenetic information. However, metaphase cytogenetic analysis remains important and is the best marker to differentiate between stroma-dependent (early) and stroma-independent (advanced) myeloma. The metaphases with normal cytogenetics obtained in most patients with myeloma are derived from the remaining normal hematopoietic elements and not from the myeloma cells. If myeloma cells are stroma dependent, they will die soon after being removed from their supporting microenvironment, while stroma-independent myeloma cells are able to grow and divide in the absence of a supporting microenvironment.<sup>148</sup>

## IMAGING STUDIES

Bone disease is present in 70 percent of patients with myeloma at diagnosis. A skeletal x-ray survey has long been considered the gold standard for assessing bone disease in myeloma. However, the sensitivity and specificity of a skeletal survey is low. At least 50 to 75 percent of trabecular bone must be lost to see a lytic lesion. The false-negative rate varies between 30 and 70 percent, which leads to significant underestimation in diagnosing and staging of patients with myeloma. The skeletal survey does not provide us with a direct image of the tumor cells, but rather with the consequence of tumor cells being present or having been present. Bone lesions in myeloma seldom heal, and in most cases, there is no healing at all or at the most there is a sclerotic rim around the bone lesion. Therefore, a skeletal survey is not a good technique to assess response to treatment or to diagnose early recurrence of myeloma. Much better imaging techniques have become available in the form of <sup>18</sup>F-fluorodeoxyglucose

positron emission tomography-computed tomography (PET/CT) and MRI. When used in combination, PET/CT scan and MRI were found to have specificity and a positive predictive value of virtually 100 percent, which is invaluable to clinicians assessing the efficacy of intensive and expensive treatment approaches with the goal to cure myeloma.<sup>149</sup>

### Magnetic Resonance Imaging

Between CT and MRI, the latter is the more specific test. A complete MRI examination should include a series of sequences to permit identification of focal and diffuse marrow involvement, including spin echo (T1 and T2 weighted), gradient echo (T2), and short T1 inversion recovery (STIR) sequences. MRI should include the axial bones with active hematopoiesis in adults (skull, spine, sternum, shoulders and upper humeri, pelvis, and upper femora). In a study from the University of Arkansas for Medical Sciences, using these parameters and including 611 myeloma patients treated uniformly with tandem transplants, 74 percent had focal myeloma lesions compared with 56 percent on skeletal survey, and 52 percent of patients with negative skeletal surveys had focal lesions on the MRI. Resolution of the MRI focal lesions after treatment occurred slowly but ultimately was seen in 60 percent of patients and conferred a superior overall survival (OS).<sup>150</sup> MRI should be used routinely for the staging, prognosis, and response assessment of patients treated with curative intent.

### <sup>18</sup>F-Fluorodeoxyglucose Positron Emission Tomography-Computed Tomography

One of the major advantages of the PET/CT scan is that it allows assessing the presence of extramedullary myeloma, which is present in 6 percent of the patients and associated with a significantly inferior OS based on two large studies. In a study of 239 patients receiving uniform therapy, the presence of more than three fluorodeoxyglucose (FDG)-avid focal lesions was associated with a significantly inferior overall and event-free survival. In contrast, complete FDG suppression prior to the first transplant conferred a significantly better outcome.<sup>151</sup> These data were confirmed in an Italian study including 192 patients.<sup>152</sup> A PET CR occurs earlier than a clinical CR and a MRI CR happens much later and less frequently.

## OTHER IMPORTANT TESTS

### $\beta_2$ -Microglobulin

The serum  $\beta_2$ -microglobulin ( $\beta_2$ m) is one of the most important prognostic factors in myeloma;  $\beta_2$ m is a small protein that associates with human leukocyte antigen class I and is almost exclusively catabolized in the kidneys. Its best-characterized function is to interact with and stabilize the tertiary structure of major histocompatibility complex (MCH) class  $\alpha$  chain. The main source of  $\beta_2$ m in the serum is membrane turnover. It reflects tumor load and renal function; however, it predicts survival irrespective of renal function and Durie-Salmon stage.<sup>153</sup> The exact underlying biologic significance of  $\beta_2$ m remains obscure, but interactions with drug resistance pathways may exist.  $\beta_2$ m in high concentrations retards the generation of monocyte-derived dendritic cells.<sup>154</sup> Serum  $\beta_2$ m and albumin levels form the basis for the International Staging System, which has major prognostic significance.<sup>99</sup>

High-risk myeloma, defined by genetic abnormalities, is independent of the International Staging System, which had only significant prognostic value in patients with a low-risk GEP and normal metaphase cytogenetics.<sup>155</sup>

### Serum Lactic Dehydrogenase Levels

The significance of high serum lactate dehydrogenase (LDH) levels in the absence of any other cause, such as liver disease or hemolytic anemia, predicting poor prognosis has long been recognized in myeloma.<sup>156</sup>

Although rarely observed in the early phase of the disease, marked elevations of LDH were detected in up to 20 percent of patients with disease progressing after vincristine, Adriamycin, and dexamethasone chemotherapy. High LDH levels were associated with hypercalcemia, elevated serum  $\beta_2$ m levels, extramedullary manifestations, plasmablastic morphology, and short OS duration, despite marked (usually very transient) antitumor responses to high-dose therapy. The same poor prognosis was noted in patients with initially normal LDH levels for whom marked LDH increments were induced by treatment, presumably resulting from tumor lysis syndrome as an indicator of rapidly proliferative myeloma.<sup>156</sup> Close correlations with plasma cell labeling index and plasmablastic morphology have been demonstrated.<sup>157</sup> Elevated serum LDH levels at diagnosis also emerged as a significant prognostic factor in an analysis of 155 newly diagnosed patients who received at least one course of high-dose therapy with ASCT, irrespective of the deletion of chromosome 13.<sup>158</sup>

## MINIMAL RESIDUAL DISEASE

Methods to assess MRD include allele-specific oligonucleotide PCR (ASO-PCR), multiparameter flow cytometry (MPC), fluorescent PCR (F-PCR), and high-throughput sequencing-based MRD assessment.

### Allele-Specific Oligonucleotide Polymerase Chain Reaction

ASO-PCR can detect one clonal cell in  $10^5$  normal cells. It remains a costly and labor-intensive assay to perform because a specific probe needs to be generated for each patient, and it is unsuccessful in approximately 30 percent of patients.<sup>159</sup> In a relatively small study of 40 patients who achieved either a CR or very good partial response after autologous transplantation and who received consolidation with bortezomib, thalidomide, and dexamethasone (VTD), eight patients achieved a molecular remission: two only in one sample and six with at least two consecutive negative samples. With a median followup of 26 months from study entry, no clinical relapses were seen in patients achieving a molecular remission, although one molecular relapse was seen, while eight clinical relapses occurred in patients not achieving a molecular remission. Importantly, VTD consolidation decreased the tumor load further after transplantation.<sup>161</sup> In contrast to earlier held opinions that molecular remissions could only be obtained with allotransplantation, this study showed that molecular remissions were also seen after autologous transplants followed by consolidation therapy with VTD.

### Multiparameter Flow Cytometry

Multiparameter flow cytometry has the advantage of being readily available and having a short turnaround time. It requires sophisticated analysis but is automated. This technique can detect one clonal cell in  $10^4$  normal cells. In the MRC Myeloma 9 study, MRD was assessed by MPC in 397 patients who received an autotransplant and 245 patients who were treated with a nontransplantation approach. A six-color panel was applied. For pretreatment samples, 100,000 events were acquired, while for the posttreatment samples a minimum of 500,000 events were analyzed. In the transplantation group, absence of MRD at day 100 after transplantation, observed in 62 percent of patients, was highly predictive of a favorable outcome (progression-free survival [PFS];  $p < 0.001$ ; OS:  $p = 0.018$ ). This outcome advantage was seen irrespective of cytogenetic findings, but more clearly in patients with adverse cytogenetics ( $p < 0.001$  vs.  $p = 0.014$ ). There was no complete agreement between immunofixation electrophoresis (IFE)-negative CR and absence of MRD by MPC. Approximately 15 percent of patients in CR still had measurable disease by MPC, while 25 percent of MRD-negative patients failed to achieve a CR by IFE. The effect of thalidomide maintenance therapy after transplantation was also assessed. The best outcomes were

seen in patients who achieved MRD negativity and were maintained on thalidomide, while the outcome was worst in patients who failed to achieve MRD negativity and did not receive thalidomide maintenance. In the MRD-positive group at day 100, 28 percent of those receiving thalidomide became MRD negative, while in those not receiving thalidomide maintenance only 3 percent became MRD negative ( $p = 0.025$ ). Finally, MRD assessment at the end of induction therapy in the non-transplantation group had no predictive value; only 15 percent of such patients became MRD negative.<sup>161</sup> It should be noted that there is large heterogeneity in assessing MRD by MPC in the United States in terms of events acquired and number of antibodies used.<sup>162</sup> If outcomes of therapies are based on MPC, it is critical that this technique becomes standardized and suitable quality controls are in place.

### Fluorescent Polymerase Chain Reaction

F-PCR can detect one clonal cell in  $10^3$  normal cells and is thus less sensitive than ASO-PCR and MPC. However, F-PCR is rapid, affordable, and easy to perform. High-molecular-weight DNA is isolated from 500  $\mu$ L of marrow. Three different multiplex PCRs are used: *IGH* D-J, *IGK* V-J, and *KDE* rearrangements. The clonal population is identified at diagnosis. Patients with a visible lack of a clonal peak identified at diagnosis were considered F-PCR negative. MRD was assessed in 130 newly diagnosed myeloma patients. The test was informative in 91.5 percent of patients. MPC was used in parallel with F-PCR. After induction, 64 patients achieved a molecular response and 66 did not. Median PFS was 61 versus 36 months ( $p = 0.001$ ). The corresponding PFS with MPC was 67 versus 42 months for MPC- and MPC+ patients ( $p = 0.005$ ).<sup>163</sup>

### High-Throughput Sequencing-Based Minimal Residual Disease Assessment

High-throughput sequencing-based MRD assessment relies on amplification and sequencing of immunoglobulin gene segments using consensus primers. It employs the *IGH*-VDJ<sub>H</sub>, *IGH*-DJ<sub>H</sub>, and *IGK* assays. The assay can detect one clonal cell in  $10^6$  normal cells and is 1 log more sensitive than ASO-PCR. The prognostic value of this test was assessed in 133 myeloma patients enrolled in the GEM myeloma trials and was compared to MPC and ASO-PCR. The test was informative in 91 percent of patients. Concordance between deep sequencing and MPC or ASO-PCR was 83 percent and 85 percent, respectively. Patients who were MRD- by deep sequencing had a significantly longer time to progression (80 vs. 31 months;  $p < 0.0001$ ). In CR patients, the time to progression remained significantly longer in MRD- patients (131 vs. 35 months;  $p = 0.0009$ ).<sup>95</sup>

There is no doubt that assessment of MRD will receive more attention in the coming years to guide the clinicians in their treatment decisions and to individualize patient care. However, it should not be automatically concluded that MRD- obtained after continuation of aggressive therapy to ultimately reach MRD- status that was not achieved after a standard aggressive approach will have the same prognostic significance as MRD- obtained with standard intensive approaches. It will also be important to establish what degree of tumor reduction has the highest prognostic significance. Extremely sensitive techniques might not necessarily provide better prognostic information than somewhat less-sensitive techniques.

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## CHAPTER 17

# ESSENTIAL MONOCLONAL GAMMOPATHY

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### SUMMARY

Essential monoclonal gammopathy is defined by two key features: (1) the presence of a monoclonal immunoglobulin or a monoclonal immunoglobulin light chain in the serum and (2) the absence of evidence for an overt malignancy of B lymphocytes or plasma cells (e.g., lymphoma, myeloma, or amyloidosis). The prevalence of essential monoclonal gammopathy depends on the demographic features in the population under study. In Americans of European descent, the prevalence increases from approximately 2 percent in individuals 50 years of age to approximately 7 percent in octogenarians. It is two to three times as prevalent in persons of African descent. The condition has been reported in association with a large variety of disorders, especially non-lymphocytic cancers. These coincidences are thought, in most cases, to be the chance concurrence of conditions that have a high prevalence in older persons. Some cases of essential monoclonal gammopathy are symptomatic because in those cases the immunoglobulin can interact with plasma proteins, blood cells, kidney, ocular structures, or neural tissue and cause serious dysfunction, for example, an acquired bleeding disorder, renal insufficiency, or an incapacitating neuropathy. In such cases, disability may be so great that attempts to remove the immunoglobulin by plasmapheresis and to suppress its production using immune or cytotoxic therapy can be warranted. Because myeloma or lymphoma may emerge at the time the monoclonal immunoglobulin is first detected, periodic evaluation of the patient is required to ascertain if essential monoclonal gammopathy is the appropriate diagnosis. Long-term followup at appropriate intervals is prudent to detect conversion from a stable, asymptomatic condition to a progressive lymphoma or myeloma, which occurs in approximately 0.5 to 1.0 percent of cases per year. In the absence of a symptomatic gammopathy or evolution to a progressive clonal gammopathy, periodic followup is all that is required.

### DEFINITION AND HISTORY

The syndrome of essential monoclonal gammopathy has two important characteristics. The first feature is a plasma immunoglobulin (Ig) or Ig light chain that has the molecular features of the product of a single clone of B lymphocytes or plasma cells: homogeneous electrophoretic migration and a single light-chain type. The second feature is the absence of evidence of an overt neoplastic disorder of B lymphocytes or plasma cells, such as lymphoma, myeloma, macroglobulinemia, or amyloidosis.

**Acronyms and Abbreviations:** CD, cluster of differentiation; HLA, human leukocyte antigen; Ig, immunoglobulin; IL, interleukin.

The observations that Bence Jones proteinuria could precede the clinical signs of multiple myeloma by many years<sup>1</sup> and that hyperglobulinemia without evidence of multiple myeloma could occur in some patients<sup>2</sup> antedated the concept of monoclonal gammopathy as a syndrome. With the more frequent clinical application of zonal electrophoresis of plasma proteins during the 1950s and 1960s, patients were discovered who had a monoclonal Ig, either without an associated disease or with diseases such as nonlymphoid cancers, infections, and inflammatory disorders, which typically are not associated with a monoclonal proliferation of B lymphocytes.<sup>3–10</sup> The presence of a monoclonal Ig or monoclonal free light chain in serum, if it is not associated with an overt neoplastic disease of lymphocytes, is referred to as *essential monoclonal gammopathy*. Several synonyms for the syndrome have been used, particularly *monoclonal gammopathy* and *benign monoclonal gammopathy*.<sup>6</sup> *Monoclonal gammopathy of unknown significance* (MGUS) has become fashionable as a designation preferable to *benign monoclonal gammopathy* because approximately one-quarter of patients eventually progress to myeloma, macroglobulinemia, amyloidosis, or a B-cell lymphoma over decades of observation.<sup>10–12</sup> The suffix “of unknown significance” was justified, it was argued, because one did not know in whom or when the progression might occur. Now, the term *monoclonal gammopathy of renal significance* has been coined, arguing that in this case the monoclonal gammopathy is of significance. However, other organs (e.g., nerves, eyes, bones), plasma proteins, and blood cells, as well as kidneys, may be injured or inactivated by a monoclonal protein (see “Functional Impairment from a Monoclonal Protein”), and we will have to have (an unnecessary) proliferation of such designations. The term *essential monoclonal gammopathy* seems best because it neither highlights a benign process nor indicates that the risks of subsequent lymphoma or myeloma are unknown; those risks are universally appreciated. It is unnecessary to assign the postscript “of unknown significance” to the numerous well-defined benign neoplasms at risk of clonal evolution and progression, such as colonic adenomatous polyps, other adenomas, uterine leiomyomas, monoclonal B-cell lymphocytosis, and clonal sideroblastic anemia. Biologically, essential monoclonal gammopathy is one of many such well-defined examples of a stable (“benign”) neoplasm with the potential to evolve through the acquisition of additional somatic mutations to a progressive neoplasm.<sup>13</sup> More is known about its significance and pathobiology than perhaps any other benign neoplasm with a risk of clonal evolution to a malignant state.<sup>12</sup> As in all diseases, the physician should understand its pathobiology and act accordingly.

Table 17–1 presents an immunologic classification of essential monoclonal gammopathy.

### EPIDEMIOLOGY

Monoclonal gammopathy can occur at any age, but it is unusual before puberty, and its frequency increases with age.<sup>14,15</sup> The frequency of a serum paraprotein using zonal electrophoresis is approximately 1 percent in persons older than age 25 years,<sup>4</sup> approximately 3 percent in those older than age 70 years,<sup>4,9</sup> and approximately 10 percent in those older than age 80 years.<sup>3</sup> A much higher prevalence of monoclonal gammopathy has been reported using more sensitive screening methods, such as isoelectric focusing or immunoblotting.<sup>16,17</sup> Prevalence rates differ in different geographic areas and have been somewhat lower in the United States (Minnesota),<sup>15</sup> Iceland,<sup>18</sup> and the Netherlands.<sup>19</sup> The frequency of monoclonal gammopathy is less in Japanese<sup>20,21</sup> and Chinese.<sup>22</sup> The prevalence rate among Africans<sup>23</sup> and Americans of African descent<sup>15,24–27</sup> is significantly greater than the rate among those of European descent in each comparative age group. Males are more frequently affected than females. Familial occurrence also has been described.<sup>28–31</sup> An increased

**TABLE 17-1.** Types of Monoclonal Immunoglobulin Synthesized by B-Cell Clone in Essential Monoclonal Gammopathy

|                                                                              |
|------------------------------------------------------------------------------|
| Serum IgG, IgA, IgM, <sup>6-12</sup> IgE, <sup>63</sup> IgD <sup>64-66</sup> |
| Serum IgG + IgA, IgG + IgM, IgG + IgA + IgM <sup>67-70</sup>                 |
| Serum monoclonal $\kappa$ or $\lambda$ light chain <sup>*71,97</sup>         |

\*Urinary monoclonal immunoglobulin light-chain excretion (Bence Jones proteinuria) may accompany serum monoclonal light chain.

incidence of monoclonal gammopathy may be associated with several occupational groups, including farmers and industrial workers, but such associations are not firmly established.<sup>32</sup> There is a higher frequency of monoclonal gammopathy among inhabitants of Nagasaki, Japan, who were younger than age 20 years when exposed to high doses of radiation from the atomic bomb detonation in 1945 than among inhabitants with lower or no exposure or older than 20 years at time of exposure.<sup>21</sup>

A significant association of ever having smoked and the risk of monoclonal gammopathy has been found in a very large and carefully analyzed study.<sup>32a</sup>

The risk of myeloma is increased in obese individuals.<sup>32b,32c</sup> An increased risk of monoclonal gammopathy with overweight and obesity was found in one study<sup>33</sup> but not in a subsequent report.<sup>32a</sup> The latter finding may indicate that the inflammatory, immune, and metabolic alterations in obesity may foster clonal progression of preexisting clones as a feature of its role in myelomagenesis (selection vs. induction).<sup>32b</sup>

## ETIOLOGY AND PATHOGENESIS

Monoclonal gammopathy can be compared with any benign tumor, such as a colonic adenomatous polyp, which can remain the same size indefinitely or undergo malignant transformation at an unpredictable future time.

Monoclonal gammopathy is caused by the proliferation of a single B lymphocyte, a plasma cell progenitor, leading to a clonal population that reaches a steady state at approximately  $1$  to  $5 \times 10^{10}$  cells. At this cell population density, marrow lymphocyte or plasma cell prevalence is indistinguishable from that of normal marrow. IgG and IgA monoclonal gammopathy arise from somatically mutated postswitch preplasma cells and may have translocations involving the Ig heavy-chain region on chromosome 14. IgM monoclonal gammopathy arises from a mutated postgerminal center lymphocyte that does not have evidence of isotype switching.<sup>34</sup> Not surprisingly, these origins determine the phenotype of the clonal B-lymphocytic diseases that may evolve. For example, IgG or IgA monoclonal gammopathy tend to evolve into myeloma or plasmacytoma (plasma cell phenotypes), and IgM monoclonal gammopathies tend to evolve into lymphomas and Waldenström macroglobulinemia (lymphocytic phenotypes).

The expanded clone secretes monoclonal Ig at a rate per cell sufficient for detection by standard tests. The clonal expansion, however, does not cause osteolysis or hypercalcemia, inhibit hematopoietic proliferation and maturation, or impair differentiation of polyclonal B lymphocytes to plasma cells. Polyclonal Ig synthesis usually is normal, and patients do not incur an increased risk of infection. The cells in the stable (benign) clone do not accumulate further and do not elaborate significant amounts of osteoclast-activating factors that are responsible for bone destruction.

Despite these significant differences from myeloma in the behavior of the neoplastic B cells, cytogenetic abnormalities akin to those seen

in myeloma may be present in plasma cells derived from patients with essential monoclonal gammopathy.<sup>34-44</sup> G-banding cytogenetic evaluation usually is normal in patients with monoclonal gammopathy, presumably related to the unavailability of cells in the cell cycle (metaphase). However, clones containing numerical abnormalities (e.g., trisomy or monosomy) and translocations have been identified with fluorescence *in situ* hybridization of interphase cells (see "Cytogenetic Analysis"). The presence of clonal cytogenetic changes does not necessarily predict clonal evolution and progression. It was initially thought that 25 to 30 percent of patients with myeloma had an identified antecedent period of essential monoclonal gammopathy that underwent clonal evolution to myeloma,<sup>40,43</sup> whereas more recent studies suggested that an antecedent period of monoclonal gammopathy may precede all patients who develop myeloma.<sup>45,46</sup> The presence of clonal cytogenetic abnormalities does not correlate with such evolution, however.<sup>34,35,42</sup> Gene-expression studies of plasma cells isolated from normal marrow and marrow from patients with essential monoclonal gammopathy have identified several hundred genes that are differentially expressed.<sup>47,48</sup> The predominant finding was a gradient of overexpression of 41 of 52 genes studied in plasma cells from normal subjects, from patients with monoclonal gammopathy, and from patients with myeloma, respectively.<sup>48</sup> In addition, myeloma patients could be stratified into those with gene-expression profiles that were more or less similar to that of essential monoclonal gammopathy. The group more similar to monoclonal gammopathy constituted approximately 30 percent of myeloma patients.

Americans of African descent have a much higher frequency of an autosomal dominant inherited risk factor for monoclonal gammopathy and myeloma. Hyperphosphorylated paratarg-7 (pP-7) results from an inability to inactivate protein phosphatase 2A, which leads to the inability to dephosphorylate p-7 at serine 17. The pP-7 carrier state is associated with an increased risk of monoclonal gammopathy and myeloma. The carrier state is more than twice as prevalent in Americans of African descent as those of European descent and is much less prevalent in Americans of Asian descent than those of European descent, a gradient similar to the incidence of monoclonal gammopathy in those populations.<sup>49</sup>

Common single nucleotide polymorphisms at 2p23.3(rs6746082), 3p22.1(rs1052501), 3q26.2(rs10936599), 6p21.33(rs2285803), 7p15.3(rs4487645), 17p11.2(rs4273077), and 22q13.1(rs877529) were associated with increased risk of myeloma. Similarly, these polymorphisms independently increased the risk of monoclonal gammopathy. Polymorphism associations were independent; risk increased with a larger number of risk alleles carried, supporting a polygenic model of disease susceptibility to monoclonal gammopathy and therefore to myeloma.<sup>50</sup>

MYD88 L265 is a somatic mutation found in approximately 50 percent of individuals with IgM monoclonal gammopathy and in more than 90 percent of patients with Waldenström macroglobulinemia. It is thought to represent an early oncogenic event in monoclonal gammopathy contributing to evolution to macroglobulinemia.<sup>51,52</sup>

The C57BL mouse provides a model of essential monoclonal gammopathy. The frequency of monoclonal gammopathy increases with age in these mice.<sup>53</sup> The gammopathy can be transferred to either irradiated or nonirradiated mice by marrow or spleen cells.<sup>54</sup> The transfer can be accomplished only during the first four consecutive transplantations, and no effect is seen on the survival of the recipient compared with that of appropriate control animals. In contrast, if mouse B-cell lymphoma or myeloma cells are transplanted into normal mice, the engraftment frequency is higher than that of B cells from mice with essential monoclonal gammopathy. Passage from the original recipient to a new recipient is unlimited. Progressive disease develops, and survival of the recipient animals is impaired. Thus, an intrinsic difference exists in the growth potential (degree of malignancy) of these B-cell clones.<sup>40</sup> The frequency



of monoclonal gammopathy increases with age, but progression to myeloma in the C57BL mouse is a rare event.<sup>55</sup> Studies in transgenic mice and their littermates replicate the increased incidence of B-cell clones and gammopathy with aging.<sup>56</sup>

Occasionally, monoclonal gammopathy is the result of exaggerated production of natural antibody by a B-lymphocyte clone.<sup>57</sup> For example, patients with cold agglutinins may have monoclonal IgM for years. A few monoclonal IgM antibodies act as rheumatoid factors and may form cryoglobulins through complex formation with IgG molecules.

## CLINICAL FEATURES

### BLOOD CELLS AND MARROW

Blood counts and the marrow examination are normal. Notably, anemia is not present, and the proportion of plasma cells in marrow is less than 10 percent. Although an increased percentage of plasma cells is the most constant morphologic feature of myeloma, the presence of cytologic atypia, as judged by frequent binucleate plasma cells and large plasma cell nucleoli, is a finding more specific for myeloma.<sup>58</sup> Quantitative microscopy of the number of marrow microvessels per high-power field, using immunohistochemistry, indicates microvessel density on average is threefold greater than in normal persons, but far less than in patients with myeloma, although some overlap with myeloma occurs.<sup>59</sup>

### CYTOGENETIC ANALYSIS

Hyperdiploidy, assessed by DNA content, is present in about half the cases, and hypodiploidy is present in approximately 10 percent of cases of monoclonal gammopathy.<sup>39</sup> Use of interphase fluorescence *in situ* hybridization has uncovered numerical chromosome abnormalities in the plasma cells of more than 50 percent of subjects. Clones containing trisomy or monosomy involving chromosomes 3, 6, 7, 9, 11, 13, 17, and 18 have been identified.<sup>35–38,41,42</sup> Deletions of 13q14 are present in about one-quarter of patients, and abnormalities involving 14q32, the site of the Ig heavy-chain genes, are present in approximately 60 percent of subjects.<sup>35–38</sup> Chromosomal changes do not appear to be correlated with progression.

### MONOCLONAL PROTEIN

Characteristically, individuals are detected by the unexpected identification of a monoclonal IgG or light chain in the serum in the absence of symptoms or signs (e.g., anemia, marrow plasmacytosis, lymph node enlargement, plasmacytoma, bone lesions, or amyloid deposits) caused by diseases associated with monoclonal proteins.<sup>6–10,57,60–68</sup> Although classically a serum Ig or urine monoclonal light chain was the standard for diagnosis, the ability to measure serum-free light chains with high specificity and sensitivity has replaced the necessity to measure urine light-chain excretion for diagnosis as the latter is less sensitive than the former.<sup>69</sup>

Monoclonal IgG gammopathy occurs in approximately 70 percent of persons and IgM and IgA in approximately 20 percent and 10 percent, respectively. A few percentage of persons may have biclonal or triclinal gammopathy (Table 17–1).<sup>6–10,12,57,60–68</sup>

Most patients with essential monoclonal gammopathy have a monoclonal protein concentration of less than 30 g/L, but exceptions occur. The diagnosis reflects the sum of (1) the monoclonal protein level; (2) the marrow plasma cell concentration (<10 percent); (3) the absence of other features of progressive plasma cell neoplasm (e.g., hypercalcemia, osteolysis, otherwise unexplained anemia, otherwise unexplained renal disease); and (4) the absence of progression on periodic long-term followup.

A developing consensus favors measurement of serum protein gel electrophoresis and serum-free light-chain levels (and the  $\kappa:\lambda$  ratio) and serum protein immunofixation electrophoresis without urinary Ig measurements to detect monoclonal gammopathies.<sup>70–72</sup> Some pathologists are still reluctant to give up urinary measurements because of the uncommon occurrence of urinary monoclonal light chains in the absence of evidence of an abnormality of serum monoclonal light chains.<sup>73</sup> Followup of these cases has not shown any clinical consequence of these uncommon false-negative serum light-chain measurements. Serum protein gel electrophoresis has a limit of detection of approximately 0.03 g/dL if the monoclonal protein migrates in the  $\gamma$ -globulin fraction. Small monoclonal proteins that migrate in the  $\alpha$ - or  $\beta$ -globulin fraction are more difficult to identify on electrophoresis. Immunofixation electrophoresis is used to confirm a monoclonal protein found on gel electrophoresis. Immunofixation electrophoresis may also detect a monoclonal protein not evident on serum protein gel electrophoresis, usually because the protein is in too low a concentration or is embedded in the normal polyclonal  $\alpha$ - or  $\beta$ -globulin peak. Monoclonal proteins identified by immunofixation electrophoresis may be transient (approximately 15 to 20 percent) and have a greater likelihood to progress to a disease if they are of an IgA or IgM isotype.<sup>74</sup> In the future, mass spectroscopy may be a more sensitive and specific method to identify monoclonal proteins.<sup>75</sup>

In individuals with a serum monoclonal IgG of less than 1.5 g/dL, especially if of an older age and with no overt end-organ abnormality, marrow examination and radiographic examination of the bones have a very low diagnostic yield and can be omitted. A presumptive diagnosis of essential monoclonal gammopathy can be made with reexamination at approximately 6 months and at appropriate intervals thereafter.<sup>76,77</sup>

### FUNCTIONAL IMPAIRMENT FROM A MONOCLONAL PROTEIN

#### Interaction with Plasma Protein or Blood Cells

Some patients have monoclonal proteins with antibody specificity directed against plasma or cell proteins, resulting in symptomatic pathophysiologic effects, such as immune hemolytic anemia,<sup>78</sup> acquired von Willebrand disease,<sup>79–81</sup> immune neutropenia,<sup>82,83</sup> and other functional manifestations<sup>84–89</sup> (Table 17–2).

#### Renal Injury

Occasional patients may have severe renal disease associated with monoclonal gammopathy.<sup>90–97</sup> The renal disease may take the form of a tubular disorder, mimicking Fanconi syndrome (glycosuria, hypouricemia, proteinuria, asymptomatic renal insufficiency)<sup>90,93</sup> or a glomerular

**TABLE 17–2.** Functional Abnormalities Associated with Essential Monoclonal Gammopathy

#### Plasma protein and blood cell disturbances

Antithyroid antibodies,<sup>78</sup> acquired von Willebrand disease,<sup>79–81</sup> immune neutropenia,<sup>82,83</sup> cryoglobulinemia,<sup>10</sup> cryofibrinogenemia,<sup>10</sup> acquired C1 esterase inhibitor deficiency (angioedema),<sup>10</sup> acquired antithrombin,<sup>84</sup> insulin antibodies,<sup>85,86</sup> antiacetylcholine receptor antibodies,<sup>87</sup> “antiphospholipid” antibodies,<sup>88</sup> dysfibrinogenemia<sup>89</sup>

#### Renal disease<sup>90–97</sup>

#### Oculopathies<sup>99–103</sup>

#### Neuropathies<sup>104–108</sup>

#### Deep venous thrombosis<sup>133,134</sup>

deposition disorder resulting from the deleterious interaction of the monoclonal Ig or a light chain and the renal parenchyma resulting in renal insufficiency.<sup>91,92,94,97</sup> The term *dangerous small B-cell clone* has been applied to a monoclonal gammopathy that can produce disease, often quite serious, without lymphoproliferation and progression of the neoplastic cell population. The principal approach to therapy is to attempt to suppress the small B-cell clone and thereby its monoclonal protein secretion with chemotherapy. Cyclophosphamide, thalidomide, bortezomib, or bendamustine are favored because they have much less renal toxicity than congeners such as melphalan or lenalidomide. A glucocorticoid and rituximab can also be useful.<sup>97,98</sup> One should also consider the possibility that the monoclonal protein may be secreted by a solitary plasmacytoma, which would be amenable to local radiation therapy. Thus, in the face of significant renal impairment, a careful evaluation, including positron emission tomography, should be considered to exclude a solitary lesion. In exceptional cases, ablative therapy with an autologous hematopoietic stem cell transplant may be considered.<sup>98</sup>

### Ophthalmic Injury

Corneal deposits of crystalline Ig<sup>99,100</sup> and a monoclonal copper binding Ig resulting in copper deposits in the eye,<sup>101,102</sup> each associated with classical monoclonal gammopathy, have led to loss of visual acuity. Remarkably, despite very high serum copper levels in the last cases, no other organ damage ensued. Iritis, vitritis, and maculopathy with loss of visual acuity have also been described in association with a monoclonal gammopathy.<sup>103</sup>

## NEUROPATHIES

### Frequency of Occurrence

A significant association exists between the incidence of neuropathies and essential monoclonal gammopathy.<sup>104–111</sup> Approximately 10 percent of patients with idiopathic neuropathy have a monoclonal Ig, a frequency about eight times that of age-adjusted healthy comparison groups.<sup>104–108</sup> The frequency of neuropathy among patients with monoclonal gammopathy varies depending on the distribution of Ig classes but is in the range of 3 to 5 percent. IgM monoclonal gammopathy has a significantly higher frequency of neuropathy than does IgG or IgA monoclonal gammopathy.<sup>104,105</sup>

### Mechanisms of Nerve Damage

Monoclonal antibodies, especially IgM, can react with peripheral nerve myelin, specifically with myelin-associated glycoprotein, glycolipids, or sulfatides.<sup>109–114</sup> Although various antinerve antibodies are present in approximately 40 percent of patients with neuropathy and IgG monoclonal gammopathy, a similar frequency has been found in such patients without neuropathy.<sup>115</sup> Neuropathy in the absence of reactivity of the monoclonal protein with nerve antigens implies other mechanisms also operate to cause nerve damage.<sup>104,106,113</sup> Deposition of monoclonal protein in the epineurium has been proposed as an alternative mechanism of nerve injury.<sup>116</sup> Also, in one report, four of 16 patients with IgG monoclonal gammopathy and neuropathy had polyclonal, not monoclonal, antibodies against neurofilament protein.<sup>113</sup> In addition, a proportion of patients develop a detectable monoclonal protein after the onset of the neuropathy, sometimes years later.<sup>115</sup>

### Signs and Symptoms

Patients with essential IgM monoclonal gammopathy and neuropathy can have dysesthesia of the hands and feet, loss of vibration and position sense, atrophy of distal muscles, ataxia, and intention tremor.<sup>111,112,114</sup> The monoclonal antibodies reactive with nerve antigens usually are of the IgM type. Serum often contains antibodies to myelin-associated

glycoprotein.<sup>104,105</sup> In contrast, patients with IgG or IgA monoclonal gammopathy usually have chronic inflammatory demyelinating polyneuropathy; a minority have sensory axonal or mixed neuropathy.<sup>105,115,117–119</sup> The neuropathy may be (1) mild with minor motor and/or sensory signs with or without mild functional impairment; (2) moderately disabling but with full range of activities; or (3) severely disabling, interfering with walking, dressing, and eating.<sup>115</sup> The course may be relapsing and remitting or progressive. IgA gammopathy is associated with dysautonomia.<sup>120</sup> The presence or absence of antibody to myelin-associated glycoprotein may have an effect on the specific nature of the neuropathic manifestations.<sup>105,106,111–114</sup>

### Diagnostic Findings

Demyelination is reflected in decreased nerve conduction velocity. Axonal loss is reflected in decreased sensory potentials.<sup>106,110,111,117–121</sup> Electromyography may show denervation of muscles.<sup>106,110</sup> Immunofluorescence studies of sural nerve or of skin biopsies may uncover Ig binding to nerve.<sup>106,111</sup> Morphologic studies of nerve biopsies may show decreased or absent myelinated fibers or axonal degeneration. A rare case of crystal formation in the epineurium has been described.<sup>122</sup>

### Management

At least seven treatment approaches have been used to ameliorate the neuropathies: (1) intravenous Ig administration; (2) glucocorticoids alone; (3) immunoadsorption of perfused blood with staphylococcal protein A; (4) plasma exchange or plasmapheresis; (5) immunosuppressive cytotoxic chemotherapy, such as cyclophosphamide, chlorambucil, or fludarabine with or without added glucocorticoids; (6) rituximab (anti-cluster of differentiation [CD] 20 antibody) to deplete B cells; and (7) high-dose cytotoxic therapy with autologous hematopoietic stem cell rescue.<sup>104–106,114,115,121,123–132</sup> In some cases, use of plasmapheresis has been followed by cytotoxic therapy in an effort to produce a sustained effect. Plasma exchange has shown benefit in a small clinical trial. The other modalities of treatment await such studies.<sup>104</sup> Response rates to each form of therapy are low, and duration of response is variable,<sup>106,115,123–128</sup> but some patients obtain coincidental significant improvement for prolonged periods. A recommendation has been made to start therapy with intravenous Ig, especially in essential monoclonal IgM-associated neuropathy, because of the relative safety of this approach.<sup>104</sup> Mild symptoms and signs may not be an indication for treatment because of the low response rate and the potential noxious effects of therapy.<sup>104</sup>

## COINCIDING DISORDERS

Monoclonal gammopathy unrelated to a clinically evident proliferation of B lymphocytes or plasma cells has been observed in association with a wide variety of conditions (Table 17–3).<sup>133–198</sup> Although they are grouped under the designation *monoclonal gammopathy with a coincidental disease*, few such reports have examined whether the coincidence is greater than expected in a control group matched for age and ethnicity, the two variables having the greatest impact on the incidence of monoclonal gammopathy. Non-B-cell malignancies, including solid tumors,<sup>3,5,6,18,175–178</sup> myeloproliferative disorders,<sup>179–186</sup> and Hodgkin and T-cell lymphomas,<sup>187–190</sup> are associated with monoclonal Ig. These relationships could result from various factors: (1) Patients with a monoclonal Ig have an increased risk of developing cancer; (2) the monoclonal Ig is an antibody against some antigen associated with the cancer; (3) the monoclonal Ig is the product of cancer cells; or (4) coincidence. The last possibility is favored by two epidemiologic studies that found the same frequency of monoclonal gammopathy in a matched control group as in cancer patients.<sup>9,18</sup> Furthermore, when the monoclonal Ig is associated with a cancer, it usually persists after successful resection of

**TABLE 17-3.** Disorders Reported in Coincidence with Monoclonal Gammopathy

|                                                                                                                                                                                                                                                                                           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Axial bone fracture <sup>135,136</sup>                                                                                                                                                                                                                                                    |
| Connective tissue diseases and autoimmune diseases: Crohn disease, cryoglobulinemia, Hashimoto thyroiditis, lupus erythematosus, myasthenia gravis, pernicious anemia, polymyalgia rheumatica, psoriatic arthritis, rheumatoid arthritis, scleroderma, Sjögren disease <sup>137-146</sup> |
| Corneal and other ocular diseases: pseudo-Kayser-Fleischer ring, <sup>243</sup> corneal gammopathy <sup>99-103,241</sup>                                                                                                                                                                  |
| Cutaneous diseases: Schnitzler syndrome, urticaria, hyperkeratotic spicules, pyoderma gangrenosum (neutrophilic dermatoses), psoriasis, scleromyxedema <sup>147-153</sup>                                                                                                                 |
| Diffuse idiopathic skeletal hyperostosis <sup>154</sup>                                                                                                                                                                                                                                   |
| Endocrine diseases: hyperparathyroidism <sup>155,156</sup>                                                                                                                                                                                                                                |
| Gaucher disease, type I <sup>157-159</sup>                                                                                                                                                                                                                                                |
| Hepatic disease: cirrhosis, <sup>145</sup> hepatitis <sup>160,161</sup>                                                                                                                                                                                                                   |
| Hereditary spherocytosis <sup>162</sup>                                                                                                                                                                                                                                                   |
| Infectious diseases: bacterial endocarditis, <i>Corynebacterium</i> species, cytomegalovirus, Epstein-Barr virus, human immunodeficiency virus, <i>Mycobacterium tuberculosis</i> , purpura fulminans <sup>19,145,163-166,209,212</sup>                                                   |
| Metabolic disease: hyperlipidemia <sup>167</sup>                                                                                                                                                                                                                                          |
| Neutropenia, chronic <sup>82,83,168</sup>                                                                                                                                                                                                                                                 |
| Osteoporosis <sup>169,171,172</sup>                                                                                                                                                                                                                                                       |
| Pituitary macroadenoma <sup>170</sup>                                                                                                                                                                                                                                                     |
| Pregnancy <sup>171</sup>                                                                                                                                                                                                                                                                  |
| Systemic capillary leak syndrome <sup>174</sup>                                                                                                                                                                                                                                           |
| Carcinomas: colon, lung, prostate, other <sup>3,5,6,175-178</sup>                                                                                                                                                                                                                         |
| Myeloproliferative diseases: acute and chronic myelogenous leukemia, <sup>179-181</sup> chronic neutrophilic leukemia, polycythemia vera <sup>182-186</sup>                                                                                                                               |
| T-cell lymphomas, Hodgkin lymphoma <sup>187-190</sup>                                                                                                                                                                                                                                     |
| After chemotherapy, radiotherapy, or marrow, kidney, or liver transplantation <sup>191-195,210-212</sup>                                                                                                                                                                                  |
| Miscellaneous diseases <sup>197-199</sup>                                                                                                                                                                                                                                                 |
| Transient, monoclonal, or oligoclonal gammopathies <sup>201-203</sup>                                                                                                                                                                                                                     |
| Factitious hyperferremia <sup>204</sup>                                                                                                                                                                                                                                                   |
| Factitious increase in C-reactive protein <sup>205</sup>                                                                                                                                                                                                                                  |
| Vitamin B <sub>12</sub> deficiency <sup>137,206</sup>                                                                                                                                                                                                                                     |

the tumor. A convincing case for an association of acute myelogenous leukemia and myelodysplasia with monoclonal gammopathy was made by one group of scientists,<sup>180-181</sup> but a large longitudinal study did not find this association.<sup>12</sup> Some observers proposed that in clonal myeloid diseases the monoclonal protein reflects B-cell lineage involvement.

Chemotherapy, radiotherapy, organ or marrow transplantation,<sup>191-196</sup> and other miscellaneous disorders<sup>5,7,10,24-26,144,145,197-199</sup> are associated with a transient or persistent monoclonal Ig (Table 17-3). The high prevalence of monoclonal proteins and associated diseases, especially after age 50 years, indicates some of these associations are coincidental. Thus, although surgical correction of hyperparathyroidism is associated with disappearance of the plasma monoclonal protein,<sup>155</sup> statistical studies of this disorder

suggest a coincidental relationship in most patients.<sup>156</sup> Gaucher disease type I has a higher-than-expected frequency of polyclonal (approximately 40 percent) and monoclonal (approximately 20 percent) gammopathy and, probably, of myeloma.<sup>157-159</sup> Elaboration of proinflammatory cytokines, growth factors, and chemokines, several involved in B-cell function, is disturbed in type I Gaucher disease. An increase of interleukin (IL) 10 and pulmonary and activation-regulated chemokine is notable. A high frequency of B-cell clonality and IgH gene rearrangements have also been described.<sup>159</sup> The administration of recombinant glucocerebrosidase therapy may decrease the occurrence and progression of gammopathies.<sup>158</sup>

In inflammatory, autoimmune, and infectious diseases, the association is viewed as an unusual expansion of a restricted population of B lymphocytes. Following marrow transplantation, the presence of oligoclonal blood B-lymphocyte populations often reflects the process of reconstitution of the B-cell population.

## ● LABORATORY FEATURES

### PLASMA AND URINARY MONOCLONAL IMMUNOGLOBULINS

The monoclonal protein usually is an IgG; however, IgM, IgA, IgD, and IgE; urinary light chains; double gammopathy involving IgA and IgG or IgM and IgA; and triple gammopathy can occur. Rare cases may have the isotype IgD or IgE (Table 17-1).<sup>12,60-63,175,200</sup> By definition, no findings other than a serum monoclonal Ig or monoclonal light chain is present, and there are no other features of a B-lymphocyte or plasma cell malignancy.

In monoclonal gammopathy of the IgG type, the concentration of monoclonal Ig usually is less than 3 g/dL. In the IgA or IgM type, the concentration usually is less than 2.5 g/dL.<sup>10,12,200</sup> However, dramatic exceptions to this rule exist. Occasional patients with essential monoclonal gammopathy have concentrations as high as 6 g/dL. Some patients have urinary monoclonal light-chain excretion (Bence Jones proteinuria) as the sole manifestation of monoclonal gammopathy.<sup>1,10</sup> The amount of urinary light chains excreted occasionally is so large (>1.0 g/day) that renal dysfunction may develop.<sup>90,93,94</sup>

Most patients with myeloma or macroglobulinemia have significantly depressed nonmonoclonal Ig levels. For example, patients with IgG myeloma usually have very low IgA and IgM concentrations and a reduced polyclonal IgG level. Patients with monoclonal gammopathy usually have normal polyclonal Ig levels, and if a decrease of their polyclonal Ig levels is present, it is usually not as severe as in myeloma.<sup>10,12,200,207</sup>

Depression of normal polyclonal immunoglobulin concentrations is considered one of several factors that may portend the likelihood of progression to a B-cell malignancy.

### OLIGOCLONAL IMMUNOGLOBULINS

Oligoclonal or monoclonal serum Ig levels have been detected with high-resolution agarose gel electrophoresis in hospitalized patients with acute-phase reactions or polyclonal hyperglobulinemia.<sup>203</sup> Oligoclonal Ig bands are frequently seen in the cerebrospinal fluid and serum of patients with a variety of neurologic conditions, especially multiple sclerosis, when the fluids are analyzed by isoelectric focusing.<sup>208</sup> Patients with AIDS have B-cell activation and aberrancies of B-cell regulation. High-resolution electrophoresis indicates most AIDS patients with advanced disease have monoclonal or oligoclonal serum Ig bands. Persons with AIDS, lymphadenopathy syndrome, or antibody to the human immunodeficiency virus also have oligoclonal or monoclonal Ig bands by standard zonal electrophoresis.<sup>164,165</sup> These monoclonal proteins are typically IgG. In about half the patients with AIDS who receive antiviral therapy, the monoclonal protein disappears by 5 years



of treatment.<sup>209</sup> Oligoclonal or monoclonal serum Ig has also been associated with Epstein-Barr virus infection and in patients after liver,<sup>210</sup> heart,<sup>211</sup> and marrow transplantation.<sup>212</sup>

## LYMPHOCYTE AND PLASMA CELL PHENOTYPES

The concentration of plasma cells in the marrow is less than 10 percent, and the incorporation of tritiated thymidine into marrow plasma cells is negligible (<1 percent) in essential monoclonal gammopathy. Marrow plasma cells in monoclonal gammopathy do not express neural cell adhesion molecule (CD56), whereas myeloma cells strongly express this surface protein.<sup>213</sup> Blood T-lymphocyte subset levels are normal in monoclonal gammopathy, whereas CD4+ T-cell levels are lower and CD8+ T-cell levels higher in myeloma and macroglobulinemia.<sup>214–217</sup> Blood B-cell concentration is normal in monoclonal gammopathy but often is decreased in myeloma patients. Clonally restricted, idiotype-positive blood B cells are characteristic of myeloma but not of monoclonal gammopathy.<sup>218</sup>

$\beta_2$ -Microglobulin is the light chain of cell surface human leukocyte antigen (HLA) molecules and is present in low concentrations in normal serum. Its concentration in serum frequently is elevated in myeloma, and the magnitude of the elevation is positively correlated with tumor mass.  $\beta_2$ -Microglobulin concentration is not elevated in essential monoclonal gammopathy.<sup>219,220</sup>

The distinction between stable essential monoclonal gammopathy and emerging (so-called larval myeloma) or low-infiltrate myeloma (so-called smoldering myeloma) with a very low tumor burden is not easily discerned except by following the patient's clinical status. This finding has not kept investigators from looking for a distinguishing test. More than 40 variables have been studied as an index for discriminating a stable (benign) from progressive (malignant) clone (Table 17–4). No single test is sufficiently sensitive and specific to be useful in an individual patient. Periodic examination of the patient is the best method for detecting the emergence of myeloma or lymphoma or a related disease. Measurement of the concentration of the serum monoclonal protein, serum polyclonal proteins, serum-free Ig light chains, serum  $\beta_2$ -microglobulin, and hemoglobin concentration at appropriate intervals is required. The marrow should be reexamined if the monoclonal protein level increases or hemoglobin concentration decreases significantly. Practical and sensitive methods for measuring bone density would be an additional useful measure of stability or progression.

## COURSE, PROGNOSIS, AND THERAPY

Longitudinal studies have reported three major patterns of outcome for patients with essential monoclonal gammopathy.<sup>10,221–223</sup> Approximately 25 percent of patients do not progress to a lymphocytic neoplasm over 25 to 30 years of observation. In this group, occasional patients experience increases in monoclonal protein concentration of up to 50 percent of their initial diagnostic value. However, these patients restabilize and do not develop signs of myeloma, macroglobulinemia, amyloidosis, or lymphoma. About half of patients die of an unrelated cause over the 25- to 30-year period of observation. The remaining 25 percent of patients develop a plasmacytoma, myeloma, amyloidosis, macroglobulinemia, lymphoma, or chronic lymphocytic leukemia over several decades of observation. The occurrence of a lymphoma or myeloma in the last group of patients continues to increase slowly without reaching a plateau. Evolution to a progressive clonal B-cell disorder has been observed more than 25 years after the diagnosis of monoclonal gammopathy. The actuarial risk of progressing to a clonal B-cell malignancy for all classes of monoclonal protein is approximately 0.5 to 1.0 percent per year

**TABLE 17–4.** Variables Used in an Attempt to Distinguish Essential Monoclonal Gammopathy from Myeloma or Lymphoma

### LYMPHOCYTES AND IMMUNOGLOBULINS

|                                                                                   |
|-----------------------------------------------------------------------------------|
| Ig $\kappa$ light-chain expression <sup>242</sup>                                 |
| Ig light chains in urine <sup>242</sup>                                           |
| Polyclonal Ig serum concentration <sup>242</sup>                                  |
| $\beta_2$ -Microglobulin or C-reactive protein serum concentration <sup>243</sup> |
| Ig-secreting cells in blood <sup>244</sup>                                        |
| Idiotype-reactive blood T lymphocytes <sup>245,246</sup>                          |
| CD4-to-CD8 lymphocyte ratio in blood or marrow <sup>214,247,248</sup>             |
| Clonally restricted B lymphocytes <sup>218,249–251</sup>                          |
| Immunofluorescence of lymphocytes <sup>215</sup>                                  |
| Natural killer cell frequency <sup>252</sup>                                      |

### PLASMA CELLS

|                                                                                                |
|------------------------------------------------------------------------------------------------|
| Frequency <sup>6,7,9,10,15,200</sup>                                                           |
| Morphology <sup>216,253–255</sup>                                                              |
| MB2 antibody reactivity <sup>256</sup>                                                         |
| Proliferative index <sup>216,217,247,253,257</sup>                                             |
| Asynchronous replication <sup>258</sup>                                                        |
| DNA content or interphase fluorescent <i>in situ</i> hybridization <sup>35,36,40,216,253</sup> |
| Gene-expression profile <sup>48,234</sup>                                                      |
| Ratio of monoclonal CD19–/CD38+/CD56++ to polyclonal CD19+/CD38+/CD56– cells <sup>259</sup>    |
| Blood or marrow concentration <sup>10,216–218,250</sup>                                        |
| J chains <sup>260</sup>                                                                        |
| Acid phosphatase <sup>261</sup>                                                                |
| Multidrug resistance expression <sup>262</sup>                                                 |
| CD19 expression <sup>263</sup>                                                                 |
| CD56/neural cell adhesion molecule expression <sup>213</sup>                                   |
| Proportion of CD19+/CD56– plasma cells in marrow <sup>264</sup>                                |
| 5' Nucleotidase <sup>265</sup>                                                                 |

### BONE INTEGRITY

|                                                      |
|------------------------------------------------------|
| Magnetic resonance imaging <sup>266,267</sup>        |
| Dual-energy x-ray absorptiometry <sup>268</sup>      |
| Histomorphometry <sup>269</sup>                      |
| Urinary pyridinium-collagen complexes <sup>270</sup> |

### MISCELLANEOUS

|                                                                                         |
|-----------------------------------------------------------------------------------------|
| Marrow microvessel density <sup>59</sup>                                                |
| Neural cell adhesion molecules <sup>271</sup>                                           |
| Serum IL-1 $\beta$ <sup>272</sup>                                                       |
| Serum IL-6, IL-10, soluble CD16, soluble IL-6 receptor, IL-1 $\beta$ <sup>273–278</sup> |
| Serum transforming growth factor- $\beta$ <sup>279</sup>                                |
| Urinary deoxypyridinoline excretion rate <sup>279</sup>                                 |
| Hemoglobin concentration <sup>8,145,175</sup>                                           |
| Mononuclear cell E-cadherin gene methylation <sup>280</sup>                             |

depending on the population studied.<sup>12,221–223</sup> IgM gammopathy usually progresses to lymphoma, macroglobulinemia, amyloidosis, or chronic lymphocytic leukemia.<sup>224,224a</sup> Although one large study found that IgM monoclonal gammopathy evolved to a progressive clonal lymphoid disorder at a rate of approximately 1.5 percent per year,<sup>225</sup> two other large studies found no significant difference in the rate of progression when patients with IgG or IgM were compared.<sup>226,227</sup> IgG or IgA monoclonal gammopathy evolves principally into myeloma, plasmacytoma, or amyloidosis.<sup>228,229</sup> Several studies have found a somewhat higher progression rate in persons with IgA monoclonal gammopathy.<sup>18,230</sup>

Patients with a higher percentage of plasma cells in the marrow, higher monoclonal Ig levels at the time of diagnosis, lower levels of polyclonal Ig, an elevated erythrocyte sedimentation rate, a more disturbed serum light-chain ratio, a lower relative proportion of CD19+ plasma cells, and more than one focal lesion in marrow as determined by whole-body magnetic resonance imaging evolve to having a progressive clonal B-lymphocyte disease more frequently.<sup>12,226–233</sup> None of these variables has the specificity and sensitivity to be highly accurate in predicting the behavior of an individual patient. Neither the plasma cell gene-expression profile nor the cell population cytogenetic findings are sufficiently specific to predict progression from a stable to an unstable clone based on current studies.<sup>41,234</sup> These findings indicate, not surprisingly, that the qualitative leap is between normal, polyclonal plasma cells (B lymphocytes) and the emergence of a monoclonal population (either stable or progressively growing) and that the distinctions between the last two states are subtle, complex, and as yet unidentified. In rare patients, the monoclonal protein appears transiently in relation to a disease (e.g., infection)<sup>199,201,202</sup> or disappears spontaneously even when not associated with a disease (clonal exhaustion).<sup>3</sup>

Generally, the diagnosis of essential monoclonal gammopathy cannot be made with certainty at the time of the initial evaluation. Periodic reexamination is required to document a stable clinical course. One of the most subtle interfaces is between essential monoclonal gammopathy and smoldering myeloma (Chap. 19). In the latter, the marrow plasma cell concentration is between 10 and 20 percent, the monoclonal protein concentration is greater than 3 g/dL, or both. There is no anemia, increase in serum calcium, or evident bone or kidney disease.<sup>238</sup> Careful observation of patients with presumed essential monoclonal gammopathy should permit identification of those who are better categorized as having smoldering myeloma. Although at this time treatment is not recommended for smoldering myeloma until progression, calls for clinical trials to assess whether early treatment may improve outcome have been made.<sup>236</sup> Therapy is not required for essential monoclonal gammopathy without a confirmed diagnosis of myeloma, macroglobulinemia, amyloidosis, or lymphoma with evidence of progressive disease. Therapy may be indicated, however, if the monoclonal protein interferes with the vital function of a normal plasma or tissue constituent, induces kidney disease, or is associated with a disabling neuropathy.

A better understanding and an ability to identify the somatic mutations that underlie the evolution of monoclonal gammopathy to a progressive and potentially fatal B-cell neoplasm may permit the application of therapy at an earlier time when curability or sustained remissions would be more frequent.<sup>237–239</sup> There is suggestive evidence that the drug metformin may decrease the risk of progression of monoclonal gammopathy to myeloma.<sup>32b</sup> Clinical trials have not been reported and the drug is not approved for that purpose.

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## CHAPTER 18

# MYELOMA

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### SUMMARY

Myeloma derives from the proliferation of a clone of malignant plasma cells that secretes a complete and/or partial monoclonal immunoglobulin protein. It originates in most, perhaps all, cases from an antecedent monoclonal gammopathy (essential monoclonal gammopathy) that progresses by clonal evolution (acquisition of additional mutations) to a malignant B-cell malignancy, often myeloma, at a rate of 1 percent per year. Myeloma cells accumulate in the marrow microenvironment, where contact with extracellular matrix and interaction with marrow accessory cells, such as osteoblasts, osteoclasts, and stromal cells, evokes cell growth and cell-survival signals and contributes to resistance to therapy. Myeloma cells show a complex genomic phenotype, with chromosomal translocations and small copy number variations that affect patient prognosis. Patients with myeloma have signs resulting from marrow infiltration (anemia), bone destruction (bone pain, pathologic fractures), excessive immunoglobulin production and deposition (renal failure and amyloidosis-related symptoms), and immunosuppression (e.g., infection). Clinical manifestations of myeloma vary as a result of the heterogeneous biology, spanning the entire spectrum from indolent to highly aggressive disease with extramedullary features. Diagnostic workup of myeloma should include serum protein electrophoresis together with immunoglobulin immunofixation; serum-free light-chain assay; a 24-hour urine collection

to quantitate urinary protein; basic blood metabolic panel, including blood counts and renal function; and marrow aspirate or biopsy with fluorescence *in situ* hybridization and cytogenetic studies. Radiographic bone survey is used to detect osteopenia and bone lesions or impending fractures. Magnetic resonance imaging (MRI) is more sensitive than the bone survey, can identify early bone disease and extramedullary disease, and can distinguish myeloma from normal marrow, helping in quantifying the extent of the disease. Positron emission tomography coupled with computed tomography (PET-CT) detects metabolically active intramedullary or extramedullary myeloma foci and lytic bone lesions. The most common staging system for myeloma is the International Staging System, based on two parameters, serum  $\beta_2$ -microglobulin ( $\beta_2$ M) and albumin; three stages are defined and correlate with patient outcome. Serum levels of  $\beta_2$ M, C-reactive protein, and number of circulating plasma cells and their labeling index are related to patient prognosis. Other prognostic factors include the presence of specific chromosomal abnormalities (deletion of chromosome 17p and loss of chromosome 1p/gain of chromosome 1q), altered gene-expression profiling, as well as MRI and PET-CT abnormalities. The introduction of the immunomodulatory drugs (IMiDs) thalidomide and lenalidomide and the proteasome inhibitor bortezomib have increased the 5-year relative survival rate to 45 percent, with some patients achieving long-term survival of more than 10 years. Melphalan-based autologous hematopoietic stem cell transplantation in combination with novel agents can achieve remarkable results in patients with good-risk myeloma; patients who are not eligible for autologous transplant have traditionally been treated with melphalan and prednisone; novel agents such as bortezomib and lenalidomide are also active and well tolerated in this population. Consolidation and maintenance regimens based on lenalidomide as a single agent or in combination with bortezomib have been evaluated to extend the duration of complete remission following autologous stem cell transplant. Finally, several novel agents can be used in the setting of relapsed/refractory disease, including new proteasome inhibitors (carfilzomib, ixazomib, and marizomib); IMiDs (pomalidomide); histone deacetylase inhibitors (vorinostat, panabinstat, and ricolinostat); and monoclonal antibodies (daratumumab and elotuzumab).

**Acronyms and Abbreviations:** auto-HSCT, autologous hematopoietic stem cell transplantation; BMSC, bone marrow stromal cell;  $\beta_2$ M,  $\beta_2$ -microglobulin; CALGB, Cancer and Leukemia Group B; CR, complete response; CrCl, creatinine clearance; CRD, carfilzomib, lenalidomide (Revlimid), and dexamethasone; CT, computed tomography; CyBorD, cyclophosphamide, bortezomib, and dexamethasone; del, deletion; DLI, donor lymphocyte infusion; EBMT, European Bone Marrow Transplant Group; ECM, extracellular matrix; EFS, event-free survival; EMP, extramedullary plasmacytoma; FISH, fluorescence *in situ* hybridization; GVHD, graft-versus-host disease; GVM, graft-versus-myeloma effect; HR, hazard ratio; IFM, Intergroupe Francophone du Myélome; IGF-1, insulin-like growth factor-1; ISS, International Staging System; LCDD, light-chain deposition disease; MG, essential monoclonal gammopathy; MGUS, monoclonal gammopathy of undetermined significance; MIP, macrophage inflammatory protein; MP, melphalan-prednisone; MPT, melphalan, prednisolone, thalidomide; MRD, minimal residual disease; MRI, magnetic resonance imaging; MTD, maximum tolerated dose; ONJ, osteonecrosis of the jaw; ORR, overall response rate; OS, overall survival; PET, positron emission tomography; PFS, progression-free survival; Rd, lenalidomide, dexamethasone; RVD, lenalidomide (Revlimid), bortezomib (Velcade), and dexamethasone; SCID, severe combined immunodeficiency; sCR, stringent complete response; SMM, smoldering (multiple) myeloma; SOP, solitary osseous plasmacytoma; TAM, tandem auto-HSCT with melphalan conditioning; TGF- $\beta$ , transforming growth factor- $\beta$ ; TTP, thrombotic thrombocytopenic purpura; VGPR, very good partial response; VTE, venous thromboembolism.

### ● DEFINITION AND HISTORY

Myeloma is a disease characterized by clonal expansion of malignant plasma cells that accumulate in the marrow, leading to anemia and associated cytopenias, hypogammaglobulinemia, osteolytic bone disease, hypercalcemia, and renal dysfunction.<sup>1</sup> Symptoms are a result of tumor mass effects, cytokine release by myeloma cells, marrow stroma or bone cells, or deposition of myeloma proteins into target organs (amyloid light-chain [AL] amyloidosis and light-chain deposition disease [LCDD]). Myeloma is part of a spectrum of diseases called plasma cell dyscrasias. These include the following conditions: essential monoclonal gammopathy (MG; also known as monoclonal gammopathy of undetermined significance [MGUS]); smoldering myeloma (Chap. 17); solitary plasmacytoma, located in the bone as an isolated lytic lesion or in the soft tissues; macroglobulinemia (Chap. 20); AL amyloidosis and LCDD (Chap. 19); and very rare disorders, such as osteosclerotic myeloma (POEMS [polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes] syndrome), Castleman disease, and heavy-chain diseases ( $\alpha$ -heavy-chain disease,  $\mu$ -heavy-chain disease, and  $\gamma$ -heavy-chain disease) (Chap. 21). Myeloma derives from cells with plasma cell morphologic features, capable of producing immunoglobulin molecules, which can be detected in the serum and/or urine by electrophoresis

and immunofixation. By definition, plasma cell dyscrasias result from the expansion of monoclonal cells, with resultant monoclonal protein secretion; however, oligoclonal and polyclonal protein abnormalities accompany some conditions, such as Castleman disease.

## ● EPIDEMIOLOGY

### INCIDENCE AND PREVALENCE

Myeloma represents the second most common hematologic cancer, accounting for 1.4 percent of all cancers and 10 percent of hematologic malignancies, with a prevalence in 2011 of 83,367 people.<sup>2</sup> The American Cancer Society has estimated that 30,280 new myeloma cases will be diagnosed in the United States in 2017, and approximately 12,590 will die of the disease in that year.<sup>2a</sup> Most patients are diagnosed between 65 and 74 years, with a median age at onset of 69 years; only 4 percent of cases occur before age 45 years. Men are affected more frequently than women (1.6:1 ratio), and individuals of African descent have twice the prevalence of myeloma as those of European descent. Conversely, individuals of Japanese and Spanish (Latino) descent have very low prevalence rates.<sup>3–5</sup> Myeloma is always preceded by a condition called MG, as has been demonstrated by long-term followup studies of a cohort of more than 70,000 banked serum samples from healthy subjects and from an independent military cohort.<sup>6,7</sup> MGUS is present in 3.2 to 4.0 percent of the general population,<sup>8</sup> developing years before the diagnosis of myeloma, at a rate of approximately 1 percent per year (Chap. 17).<sup>9</sup>

### GENETIC PREDISPOSITION

The etiology and the mechanisms of myeloma progression are still largely unknown.<sup>1</sup> Several epidemiologic reports have demonstrated an increased risk of myeloma or MG (up to fourfold) in first-degree relatives of individuals affected by plasma cell dyscrasias.<sup>10</sup> Moreover, myeloma is associated with an increased risk of prostate cancer, melanoma, non-Hodgkin lymphoma, and chronic lymphocytic leukemia.<sup>11</sup> More than 100 myeloma families have been described from different geographic areas<sup>12–14</sup>; in one family, a germline mutation of the *CDKN2A* (*p16*) gene together with loss of heterozygosity of the other allele was identified as a rare low-penetrance genetic risk.<sup>13</sup> Genome-wide association studies have identified seven single nucleotide polymorphisms (SNPs) at chromosomes 2p23.3, 3p22.1, 3q26.2, 6p21.33, 7p15.3, 17p11.2, and 22q13.1 that are associated with risk of myeloma. The identified genes (*DNMT3A*, *ULK4*, *TERC*, *PSORS1C1*, *CDCA7L*, *DNAH1*, *TNFRSF13B*, and *CBX7*) have never been validated as myeloma-driver genes.<sup>15,16</sup> These same SNPs have also been independently associated with development of MG, with the risk increasing with the number of alleles carried.<sup>17</sup> Moreover, the presence of hyperphosphorylated paratarg-7 (pP-7) carrier status has been reported as an autosomal dominantly inherited risk factor for MG and myeloma in a European population and in MG/myeloma cases in African Americans.<sup>18,19</sup>

### LIFESTYLE AND OCCUPATIONAL FACTORS

Some epidemiologic studies, including a cohort study of Swedish and Finnish twins and a meta-analysis,<sup>20,21</sup> have shown an association between high body mass index and risk of myeloma.<sup>22,23</sup> Specifically, obese individuals have higher levels of cytokines, such as interleukin (IL) 6 and insulin-like growth factor (IGF) 1, which also are produced by adipocytes and are potent growth factor for myeloma cells.<sup>24</sup> No consistent associations have been observed with any particular diet, alcohol consumption, or smoking.<sup>25</sup> Occupational exposure to pesticides, organic solvents (benzene, petroleum derivatives, styrene), or chronic

radiation have been alleged to be associated with myeloma in some studies,<sup>25,26</sup> but refuted by others. Furthermore, the use of thorium dioxide (thorotrast), a contrast medium used in the 1950s for angiography, increases the risk of plasmacytomas up to fourfold.<sup>27</sup> Exposure to acute radiation, as in atomic bomb survivors increases the overall rate of MG or myeloma after 15 to 20 years<sup>28</sup> and accelerates MG transformation to myeloma.<sup>29</sup> People exposed to fresh wood, wood dust, or working in sawmill factories had an increased risk of myeloma in some studies.<sup>30,31</sup> Finally, associations between myeloma risk and autoimmune diseases (especially rheumatoid arthritis<sup>32,33</sup> or pernicious anemia<sup>34</sup>) or infections (HIV and hepatitis C<sup>35,36</sup>) have been proposed, based on a retrospective study among American male military veterans,<sup>37,38</sup> suggesting an immune-mediated mechanism for malignant transformation. Human herpes virus 8 DNA sequences responsible for Kaposi sarcoma and Castleman disease pathogenesis<sup>39</sup> have been reported by some investigators in marrow dendritic cells of myeloma patients, although other studies suggested that this is an epiphenomenon.<sup>40,41</sup>

## ● ETIOLOGY AND PATHOGENESIS

### CELL OF ORIGIN

Myeloma cells derive from postgerminal-center marrow plasmablasts/plasma cells (Fig. 18–1). Myeloma cell immunoglobulin heavy-chain (IGH) variable genes present somatic mutations in the absence of intraclonal variation or ongoing somatic hypermutation, indicating antigen-contact selection in the germinal center.<sup>42,43</sup> The existence of a myeloma stem cell, that is, a precursor with self-renewal capacity, has been proposed for a long time, given myeloma's low proliferative index

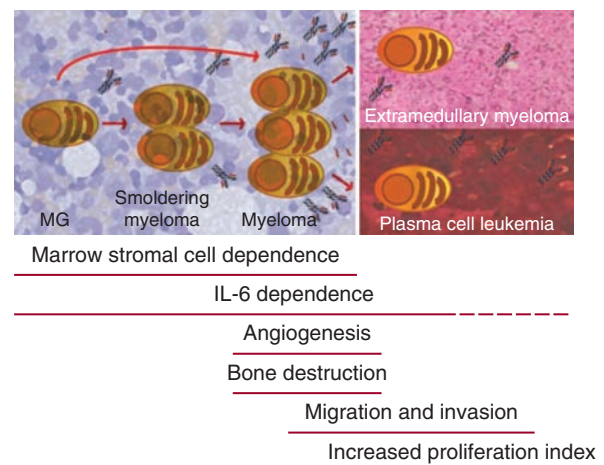


**Figure 18–1.** Myeloma stages, from essential monoclonal gammopathy (MG) to plasma cell leukemia. Myeloma evolves from a benign condition called essential monoclonal gammopathy (or monoclonal gammopathy of undetermined significance), with an annual rate of progression of 1 percent. In some patients, a stage called smoldering myeloma is sometimes evident, where there is a higher number of monoclonal plasma cells in the marrow, but still absence of symptoms. At early stages during the so-called intramedullary phase, myeloma cells are totally dependent on marrow microenvironment to survive and on interleukin (IL) 6 and other cytokines. During progression, myeloma cells can acquire the capability of growing without microenvironmental support and localize to other tissues (extramedullary disease) or circulate in the blood (secondary plasma cell leukemia). Active myeloma is characterized by onset of angiogenesis and bone lytic lesions, in contrast to MG or smoldering myeloma; during late stages, there is an increase in migration and invasion capabilities, as well as high proliferative rates.



and clonogenic efficiency. However, this remains a matter of debate.<sup>44</sup> Myeloma is a multistep process, always preceded by an MG phase.<sup>6,7</sup> MG cells share several similarities with myeloma, including a similar prevalence of hyperdiploidy and of the three primary IGH rearrangements [t(6;14), t(11;14), and t(14;16)]. However, chromosome 13 deletions, *RAS* mutations, and non-immunoglobulin (Ig) locus-associated *MYC* translocations are more frequent in myeloma.<sup>43</sup> Indeed, the development of myeloma seems to necessitate an immortalizing event, such as a primary IGH translocation, an oncogene activation, or deregulation of a tumor suppressor, to occur in the germinal center during the switch recombination or somatic hypermutation, resulting in uncontrolled expansion of a long-lived plasmablast/plasma cell.<sup>42</sup> In early stages, myeloma cells are dependent on the growth support provided by bone marrow stromal cells (BMSCs) (intramedullary phase) but can become independent of their medullary environment at late stages (plasma cell leukemia). However, 15 to 70 percent of newly diagnosed myeloma patients, using conventional morphology techniques<sup>45–51</sup> or multiparametric flow cytometry,<sup>52</sup> have circulating clonotypic myeloma cells (circulating tumor cells [CTCs]) in their blood, suggesting the presence of a “metastatic”/dissemination process that disseminates the disease hematogenously.<sup>53</sup> Moreover, the presence of CTCs in MG is a risk factor for myeloma progression,<sup>54,55</sup> as well as a poor prognostic factor in newly diagnosed or relapsed/refractory myeloma patients.<sup>56,57</sup> Myeloma CTCs share a similar phenotype to marrow myeloma cells but are more quiescent, have better *in vitro* clonogenic capacity, and have lower expression of integrin and adhesion molecules (including CD138), making them less dependent on marrow niches.<sup>58</sup>

## GENOMIC ALTERATION

### Abnormal Karyotype and Common Translocations

Myeloma is a heterogeneous disease with a complex genetic landscape, characterized by several numerical and structural aberrations, including abnormal karyotypes, chromosomal translocations, and copy number changes (Fig. 18–2 and Table 18–1).

Traditionally, myeloma patients have been divided into two subgroups: hyperdiploid cases with more than 46 but less than 76 chromosomes (34 to 60 percent of myeloma) and nonhyperdiploid cases, which include individuals with a hypodiploid (up to 44 to 45 chromosomes), pseudodiploid (44/45 or 46/47 chromosomes with gains or losses), and near-tetraploid karyotype.<sup>59–61</sup> Hyperdiploid patients, normally IgG kappa type with bone involvement, show gains of odd-numbered chromosomes, including trisomies of chromosomes 15, 9, 5, 19, 3, 11, 7, and 21 (including by decreasing frequency), and have a favorable prognosis that can however be affected by the concomitant presence of additional abnormalities, such as chr11 or chr1q gains or chr13 loss.<sup>62,63</sup> Fluorescence *in situ* hybridization (FISH) analysis is employed to detect five major primary IGH translocations in myeloma,<sup>64</sup> which occur more frequently in nonhyperdiploid patients (85 vs. <30 percent).<sup>65</sup> Primary translocations are caused by errors during normal DNA recombination in isotype class switching of terminally differentiated B cells. Conversely, IGH translocations involving chromosome 8p24 and 11q13 (called secondary translocations) result from errors in somatic hypermutation processes.<sup>42</sup> All of the translocations induce increased constitutive expression of specific oncogenes by their juxtaposition to immunoglobulin enhancer elements. The most frequent translocation (20 percent of cases) is t(11;14)(q13;q32),<sup>66–68</sup> leading to upregulation of cyclin D1, a crucial promoter of G<sub>1</sub>-to-S transition via cyclin-dependent kinase (CDK) 4 or CDK6.<sup>69,70</sup> Rarely, cyclin D2 and cyclin D3 can be rearranged via t(12;14) (<1 percent) or t(6;14) translocations (2 percent of myeloma patients), respectively.<sup>71</sup> Even in the absence of translocations, cyclins D1, D2, and D3 are often upregulated, creating specific patient

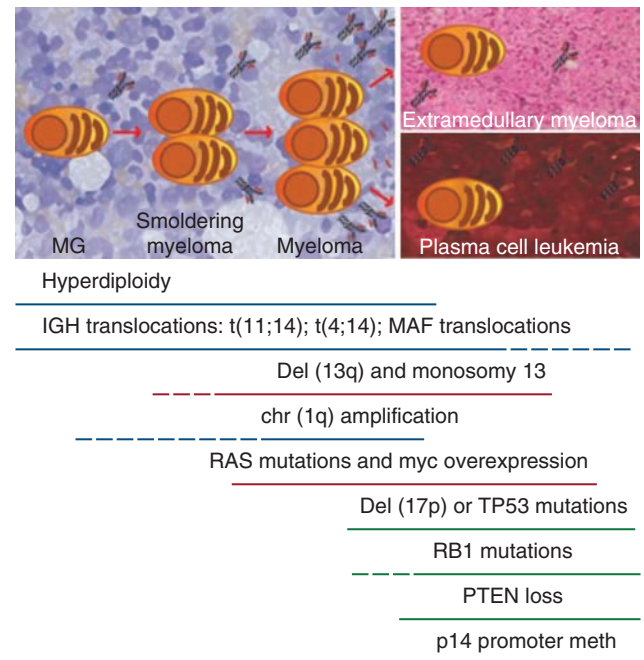


**Figure 18–2.** Genomic aberrations, including karyotype abnormalities, chromosomal translocations, and copy number variations in essential monoclonal gammopathy (MG), myeloma, and plasma cell leukemia. Myeloma cells are characterized by several genomic aberrations, which combine differently in distinct patients. Hyperdiploidy and immunoglobulin heavy-chain (IGH) translocations [t(11;14), t(4;14) and *MAF* translocations] are already present in the MG phase, a benign condition that can evolve to active myeloma at a rate of 1 percent per year. These abnormalities are not considered driver events in myeloma. Conversely, several groups have proposed other aberrancies, such as *MYC* translocations and increased *MYC* mRNA levels or *RAS* mutations as transforming events, because they are rare in MG and smoldering myeloma but common in myeloma. Also, chromosome gains and losses, including deletion of chromosome 13q or monosomy 13, deletion of chromosome 1p, and amplification of chromosome 1q21, are seen more frequently in active myeloma, even though their role in myeloma progression is still not totally elucidated. Deletion of chromosome 17p or *TP53* mutations are rare at diagnosis but present in advanced/relapsed settings, being associated with reduced response to treatment and unfavorable patient outcomes. The acquisition of independence from support by the marrow microenvironment is a feature of advanced myeloma, possibly leading to plasma cell accumulation in various organs (extramedullary disease) or in the blood (plasma cell leukemia). *PTEN* losses, methylations of p14 promoter, and *RB1* inactivations are reported more frequently in plasma cell leukemia, suggesting a role in the development of extramedullary growth.

subgroups with different prognoses.<sup>72</sup> Specifically, the CD-1 subgroup (cyclin D1-high) responds well to treatment and has an increased frequency of early relapse but also has an excellent long-term survival, while the CD-2 subgroup (cyclin D3-high) exhibits a lymphoplasmacytoid morphology. Translocation t(4;14), a poor prognostic factor, pairs *MMSET/WHSC1*, a nuclear SET DOMAIN protein with *FGFR3* (fibroblast growth factor receptor), an oncogenic tyrosine kinase receptor in 15 percent of patients, often in association with chromosome 13 abnormalities.<sup>73–76</sup> *MMSET* is an H3K4-, H3K27-, H3K36-, and H4K20-specific histone methyltransferase that causes global changes in chromatin status, favoring myeloma cellular and clonogenic growth, adhesion, and tumorigenicity,<sup>75</sup> while *FGFR3* promotes myeloma cell proliferation via *RAS*-*MAPK* (mitogen-activated protein kinase) and

**TABLE 18-1.** Common Genomic Aberrations in Essential Monoclonal Gammopathy, Myeloma, and Plasma Cell Leukemia\*

| Genetic Lesion                            | MG    | Myeloma | Plasma Cell Leukemia |
|-------------------------------------------|-------|---------|----------------------|
| Hyperdiploidy                             | 50%   | 60%     | 20%                  |
| t(11;14)                                  | 5–10% | 20%     | 25–60%               |
| t(4;14)                                   | 2–3%  | 15%     | 15–25%               |
| MAF translocations                        |       | 5%      | 15–35%               |
| Del(13q)/monosomy 13                      | 20%   | 50–60%  | 60–80%               |
| Del(1p)                                   | 4%    | 7–40%   |                      |
| Chr 1q21 amplification                    |       | 40%     | 70%                  |
| Cyclin D dysregulation                    | 60%   | 80%     |                      |
| RAS mutations                             | <5%   | 30–50%  | 30%                  |
| FAM46C, DIS3                              |       | 10–21%  |                      |
| NF-κB-activating mutations and CNVs       |       | 15–20%  |                      |
| IGH MYC rearrangements                    | 1–2%  | 15%     | 30–50%               |
| UTX deletions and mutations               |       | 30%     |                      |
| TP53 inactivations [mutations + del(17p)] | 5%    | 10–20%  | 20–80%               |
| p18 and/or Rb inactivation                |       | <5%     | 25–30%               |
| p14 promoter methylation                  |       | <5%     | 25–30%               |
| PTEN loss                                 | 0%    | <2%     | 8–33%                |

CNV, copy number variant; IGH, immunoglobulin heavy chain; MG, essential monoclonal gammopathy; NF-κB, nuclear factor kappa B; Rb, retinoblastoma tumor-suppressor protein.

\*Myeloma is a multistep process, progressing from an indolent MG stage, to overt myeloma, to plasma cell leukemia. Hyperdiploidy and IGH translocations [t(11;14), t(4;14) and MAF translocations] are present at similar rates in MG and myeloma. Conversely, MYC secondary rearrangements, deletion 13p, chromosome 1 abnormalities, and RAS mutations are more common in active myeloma, and they have been postulated as driver myeloma events. Plasma cell leukemia shows distinct abnormalities, including p14 promoter methylation and PTEN losses. Frequencies of common genomic aberrancies in plasma cell dyscrasias are reported. Blank spaces are left in case of unknown data.

STAT (signal transducer and activator of transcription) pathways.<sup>77</sup> In addition, activating *FGFR3* mutations, mutually exclusive with RAS mutations, have also been reported in a small fraction of patients.<sup>78,79</sup> MAF translocations, which include t(14;16) overexpressing *c-MAF*, t(14;20), which deregulates *MAFB*, and t(8;16) involving *MAFA*, are relatively rare (5 percent, 2 percent, <1 percent of cases, respectively) but associated with poor prognosis.<sup>80</sup> *c-MAF*, *MAFA*, and *MAFB* are all transcription factors involved in proliferation, responsiveness to IL-6, and BMSC-MM (multiple myeloma) adhesion,<sup>81</sup> promoting cell-adhesion-mediated drug resistance (CAM-DR), via integrin  $\alpha_5$ /E-cadherin interactions.<sup>82</sup> The prevalences of these three primary IGH rearrangements

[t(6;14), t(11;14), and t(14;16)] are similar in MG, indicating the need for additional transforming events to precipitate active myeloma.<sup>83–85</sup>

### Copy Number Alterations in Myeloma

Array comparative genomic hybridization (aCGH) analysis demonstrates numerous copy number alterations (CNAs) in myeloma cells. Deletion of chromosome 13, deletion of chromosome 17p13, and amplification of chromosome 1q21 are genomic aberrations associated with poor prognosis in myeloma patients.<sup>86,87</sup> Deletion of chromosome 13 affects 50 to 60 percent of newly diagnosed myeloma, is more frequent in the nonhyperdiploid group (>70 percent) in comparison to the hyperdiploid group, and often co-occurs with t(4;14) or t(14;16) translocations.<sup>88</sup> Among other genes, *RB1* and the miRNA-15a/16-1 cluster are deregulated in this context and may play a role in myeloma pathogenesis. Despite being traditionally associated with a poor prognosis, the adverse impact of isolated chromosome 13 deletion is now controversial<sup>89</sup> in view of the good response of such patients to bortezomib-based regimens and the close association of del13 with the t(4;14)(p16;q32) and hypodiploid karyotype.

Deletions of chromosome 17p involving the *TP53* locus are rare in newly diagnosed myeloma (5 to 10 percent), more common in relapsed and refractory cases (20 to 40 percent), and inevitably associated with negative prognosis, causing early relapse in patients treated with or without autologous stem cell transplantation (SCT). Mutations in *TP53* are also often present on the second allele.<sup>90,91</sup> Despite having an unfavorable prognosis, regimens containing bortezomib can increase the median progression-free survival (PFS) and 3 years of patients with *TP53* mutations (17 to 69 percent [ $p = 0.028$ ]) in comparison to non-bortezomib-containing regimens, as shown by the hemato-oncologie voor volwassenen nederland 65/german multicenter myeloma group high-dose melphalan 4 trial (HOVON-65/GMMG-HD4 trial).<sup>92</sup> 1q21 amplification is detected by FISH in approximately 40 percent of newly diagnosed myeloma and in 70 percent of relapsed myeloma and negatively affects overall survival (OS), with a cumulative effect based on the number of 1q21 locus copies.<sup>93</sup> Possible target genes of this lesion are *CKS1B*, a protein that regulates cyclin-dependent protein kinases, *PSMD4*, a proteasome subunit modulating response to bortezomib treatment, *MCL1*, or *BCL9*.<sup>93–95</sup> Interestingly, a reported jumping translocation of 1q12 (JT1q12) can have a receptor chromosome *TP53* genomic locus, causing simultaneous gain of 1q21 and deletion of 17p.<sup>96</sup> In addition, deletions of 1p, present in 7 to 40 percent of patients, are linked to reduced PFS and OS despite autologous SCT.<sup>97–99</sup> *TP73*, *LAPTM5*, *CDKN2C*, a CDK inhibitor that interacts with CDK4 or CDK6 to regulate G<sub>1</sub>/S phase, *MTF2*, *TMED5*, and *FAM46C* are candidate genes of the deletion. *FAM46C* loss or *FAM46C* mutations (evident in 15 percent of patients) are especially associated with shortened survival (median OS 25.7 months vs. 51.3 months,  $p = 0.004$ ).<sup>97,100</sup> The biologic function of *FAM46C* is uncertain, although some data suggest it is related to mRNA stabilization. Other mutations include *MYC* rearrangements involving unbalanced translocations and insertions, small duplications, amplifications, and inversions on chromosome 8p24<sup>101–104</sup>; homozygous deletions of 11q22 locus, resulting in loss of *YAPI*, *BIRC3*, and *BIRC2* genomic region<sup>105–107</sup>; chromosomes 4, 14, and 16 aberrations that disrupt *FGFR3*, *WFOX*, and *CYLD*; deletions or amplifications of chromosome 6; and homozygous deletions of Xp11.2 locus,<sup>62,63</sup> involving *UTX*, a histone H3 lysine 27 (H3K27) demethylase, which is also mutated in 10 percent of myeloma,<sup>100</sup> are also common. The “purpose” of these multiple plasmin inhibitors is to guard against premature plasmin activation and subsequent degradation of fibrinogen until intravascular fibrin begins to appear.

**Somatic Mutations and Interclonal Diversity** Myeloma evolves in a stepwise process, transforming from MG to smoldering myeloma to overt myeloma, where mutations accumulate, conferring either a

growth advantage (driver mutations) or functional irrelevance (passenger mutations). So far, more than 300 myeloma patient DNA samples have been sequenced using whole-genome or whole-exome sequencing approaches.<sup>100,108–113</sup> Specifically, 11 genes are commonly mutated in myeloma, reaching a standard significant threshold.<sup>112</sup> Five of them (*KRAS*, *NRAS*, *FAM46C*, *DIS3*, and *TP53*) are relatively frequent.<sup>100,108</sup> Approximately 30 to 50 percent of newly diagnosed patients have *RAS* mutations at codons 12 or 13,<sup>82,114–117</sup> often in association with t(11;14), but mutually exclusive with t(4;14), which constitutively activates the MAPK pathway via *FGFR3* upregulation.<sup>115,118,119</sup> Because *RAS* mutations are rare in MG, they are considered a possible driver to progression.<sup>120</sup> Conversely, *TP53* mutations are a late event in myeloma,<sup>91,121,122</sup> are an independent poor prognostic factor,<sup>123</sup> and are associated in one-third of patients with concomitant hemizygous deletion of chromosome 17.<sup>90,124</sup> Mutations in *FAM46C* and *DIS3*, genes possibly involved in RNA processing, are present in 10 to 21 percent of patients, often coupled with loss of heterozygosity in the remaining allele. Other significant genes are *BRAF* (4 percent of patients), *TRAF3*, *CYLD*, *RB1*, *PRDM1*, and *ACTG1*. *TRAF3* and *CYLD* mutations, together with homozygous deletions in *BIRC2/BIRC3*; *NIK* overexpression and mutations in other genes (*CARD11* and *MYD88*) contribute to constitutive activation of the NF- $\kappa$ B pathway.<sup>100,105,107,112</sup> Genes involved in (a) protein homeostasis, (b) the unfolded protein response, (c) lymphoid/plasma cell development, such as *PRDM1* involved in plasmacytic differentiation, and (d) *XBP1*, *IRF4*, *LRRK2*, *SP140*, and *LTB* form a cluster of genes mutated in myeloma. Other recurrent mutated genes are *ROBO1*, a transmembrane receptor involved in  $\beta$ -catenin and MET signaling; *EGR1* transcription factor; *FAT3*, a transmembrane protein belonging to the cadherin superfamily; and histone-modifying genes (*MLL*, *MLL2*, *MLL3*, *WHSC1/MMSET*, *WHSC1L1*, and *UTX*, among others).<sup>100,108,112</sup> Plasma cell leukemia patients possess different aberrancies, including p14<sup>ARF</sup> promoter methylation, *PTEN* loss, *RB1* mutations, and higher rates of *TP53* mutations and deletions.<sup>125</sup> Finally, a novel intriguing concept is the idea of intratumor heterogeneity in myeloma, where different subclones can emerge and become predominant following different mechanisms of evolution, including linear, branching, parallel, or convergent evolution.<sup>108,126</sup> Clonal diversity is indeed a fundamental process akin to Darwinian selection, favoring cancer progression and adaptation to therapy. Next-generation sequencing analyses show that most patients have a subclonal structure at diagnosis, with one predominant clone and several others that can reappear at different stages or following treatment.<sup>108,110,111</sup> Gene-expression profiling of myeloma cells helps categorize patients into distinct subgroups<sup>127,128</sup> and can predict therapeutic responsiveness to specific drugs, although the role of specific genetic signatures in risk stratification is still under debate.<sup>129</sup>

## ROLE OF MARROW MICROENVIRONMENT IN MYELOMA

The marrow microenvironment is composed of extracellular matrix (ECM) proteins, such as fibronectin, collagen, laminin, and osteopontin; and cells, including hematopoietic stem cells, BMSCs, and endothelial cells, as well as osteoclasts and osteoblasts (Fig. 18–3).<sup>130–133</sup> Myeloma cells physically interact with ECM proteins and accessory cells to gain growth, survival, and drug resistance advantages. Among others, CD44, very-late antigen 4 (VLA4), neuronal adhesion molecule (NCAM), intercellular adhesion molecule (ICAM) 1, and syndecan 1 (CD138) mediate the adhesion of myeloma cells to the marrow and ECM, activating signaling pathways, such as nuclear factor kappa B (NF- $\kappa$ B), to obtain CAM-DR to conventional chemotherapy.<sup>134,135</sup> In particular, CD138 (syndecan-1) is a transmembrane heparan sulfate bearing proteoglycan, expressed during the plasma cell stage of B-cell

maturation, that can bind to type I collagen, inducing expression of metalloproteinases and promoting bone resorption and invasion.<sup>136</sup> Moreover, CD138 can be shed in the ECM, trapping growth-promoting and proangiogenic cytokines.<sup>137,138</sup> Increased soluble CD138 levels correlate with tumor burden and poor outcomes.<sup>139</sup> The SDF-1/CXCR4 axis regulates not only specific homing of myeloma cells to the marrow but also mobilization or marrow egress, being possibly accountable for the multifocal marrow localization and blood circulation of myeloma cells.<sup>140,141</sup> Moreover, other chemokine receptors, such as CXCR3, CCR1, CCR2, and CCR5, can be expressed by myeloma, conferring different migration capabilities to medullary and extramedullary cells.<sup>142</sup> Accessory cells (BMSCs, endothelial cells, osteoclasts, and osteoblasts) secrete factors that include IL-6,<sup>143–146</sup> IGF-1,<sup>147–149</sup> vascular endothelial growth factor (VEGF),<sup>150,151</sup> tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ),<sup>152</sup> fibroblast growth factor (FGF),<sup>153</sup> stromal cell-derived factor 1 $\alpha$  (SDF-1 $\alpha$ ),<sup>141</sup> and B-cell activating factor (BAFF)<sup>154</sup>; all are capable of promoting expression of survival factors such as NF- $\kappa$ B,<sup>155</sup> IL-6 and other survival signals also induce phosphatidylinositol 3'-kinase (PI3K)/AKT,<sup>156,157</sup> STAT3, and MAPK signaling (see Fig. 18–3).<sup>133</sup> BMSCs, myeloma cells, and osteoclasts also secrete growth factors and cytokines such as VEGF, basic fibroblast growth factor (bFGF), and IL-8 to promote marrow angiogenesis, which increases delivery of oxygen and nutrients to myeloma cells. Moreover, the same endothelial cells produce growth factors (IL-6 and IGF-1), to favor plasma cell survival<sup>150,151,158,159</sup> and express deregulated genes important for ECM and bone remodeling, cell adhesion, migration, and resistance to apoptosis.<sup>160</sup> The degree of marrow angiogenesis, as assessed by microvessel density (MVD), is higher in active myeloma compared with MG<sup>161</sup> and is also related to myeloma proliferation and infiltration, negatively affecting patient prognosis.<sup>162</sup> Also, lymphoid and myeloid cells are part of marrow microenvironment and can modulate myeloma survival. Myeloid cells, such as macrophages, mast cells, and neutrophils, control both pro- and antiinflammatory responses and regulate antigen presentation.<sup>163</sup> For instance, a specific group of myeloid cells, named myeloid-derived suppressor cells (MDSCs),<sup>164</sup> are highly represented in the marrow of myeloma individuals in comparison to healthy persons and increase with disease progression, facilitating tumor development, growth, and immune escape by blocking T-cell (CD8+ T and natural killer [NK] T) antitumor immune responses.<sup>165</sup>

## BONE METABOLISM

The presence of osteolytic bone lesions, bone pain, increased risk of pathologic fractures, and generalized bone loss (or osteoporosis) is a well-defined feature of myeloma.<sup>166</sup> Indeed, as myeloma burden increases, an imbalance between osteoblast and osteoclast activities ensues, with suppression of bone formation by osteoblasts and uncoupled activation of osteoclasts (Fig. 18–4).<sup>167–169</sup> The ligand for receptor activator of NF- $\kappa$ B (RANKL) binds to the RANK (receptor activator of NF- $\kappa$ B) receptor to stimulate osteoclast differentiation, formation, and survival<sup>170</sup>; myeloma cells produce RANKL and upregulate RANKL expression in BMSCs and osteoblasts via direct contact interaction, signaling induction<sup>171–173</sup> or production of IL-7. Moreover, they promote suppression of osteoprotegerin (OPG),<sup>174–176</sup> a decoy receptor that normally prevents RANK-RANKL interaction<sup>177,178</sup> via soluble factors, integrin  $\alpha_v\beta_1$ -vascular cell adhesion molecule (VCAM) 1 interaction,<sup>179</sup> production of Dickkopf-1 (DKK1),<sup>180</sup> or inactivation by syndecan-mediated internalization into myeloma cells.<sup>181</sup> Interestingly, OPG levels are decreased in the serum of myeloma patients and correlate with lytic bone lesion development<sup>182</sup>; a high RANKL-to-OPG ratio is associated with worse prognosis.<sup>183</sup> Recombinant OPG constructs, soluble RANK, OPG peptidomimetics,<sup>175,178,184,185</sup> and an anti-RANKL antibody, denosumab,<sup>186–189</sup> have been developed to modulate the RANKL/OPG axis



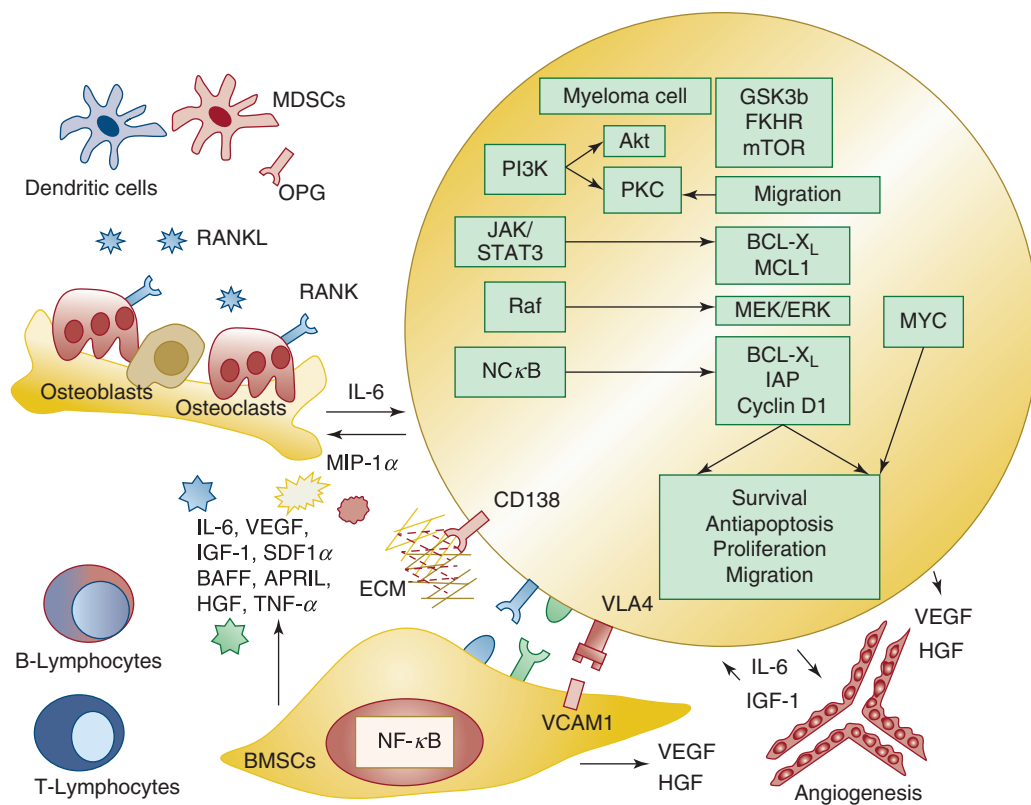


**Figure 18–3.** Myeloma cell interaction with extracellular matrix (ECM) and accessory cells in the marrow. Myeloma cells require support from bone marrow stromal cells (BMSCs) during early stage disease. Adhesion between myeloma cells and BMSCs favors myeloma cell survival, growth, and migration via release of cytokines (IL-6, VEGF, IGF-1, SDF1 $\alpha$ , BAFF, APRIL, HGF, TNF- $\alpha$ ) from both myeloma cells and BMSCs. Among others, extracellular signal-regulated kinase (ERK); Janus kinase 2 (JAK2)–signal transducer and activator of transcription 3 (STAT3); phosphatidylinositol 3'-kinase (PI3K)–Akt; nuclear factor- $\kappa$ B (NF- $\kappa$ B) and MYC are constitutively active in myeloma, promoting transcription or activation of important targets, including cytokines (IL-6, IGF-1, VEGF); antiapoptotic proteins (BCL-XL, IAP, MCL1); cell-cycle modulators (cyclin D1); and proteins involved in migration, invasion, and autophagy. NF- $\kappa$ B activation in both myeloma and BMSCs upregulates adhesion molecules (VCAM1, VLA4) to promote reciprocal binding. Proangiogenic factors, including VEGF and HGF, are released from myeloma cells, BMSCs, and marrow endothelial cells to promote neoangiogenesis and increase delivery of oxygen and nutrients to tumor cells. Cells from the innate and adaptive immune response, including B lymphocytes, T lymphocytes, dendritic cells, and myeloid-derived suppressor cells, are also modulated by myeloma cells, creating an immunosuppressive microenvironment that promotes tumor survival and reduces antigen-presenting capabilities. Receptor activator of NF- $\kappa$ B ligand (RANKL) and MIP-1 $\alpha$  are produced by BMSCs and myeloma cells and trigger osteoclast activation via RANK receptor. Osteoprotegerin (OPG), a decoy receptor for RANKL secreted by osteoblasts and BMSCs to block RANKL–RANK ligand interaction and inhibit osteoclastogenesis, is reduced in myeloma patients. APRIL, a proliferation-inducing ligand; BAFF, B-cell activating factor; BCL-XL, B-cell lymphoma-extra large; FKHR, forkhead in rhabdomyosarcoma; GSK3B, glycogen synthase kinase 3 beta; HGF, human growth factor; IAP, inhibitors of apoptosis proteins; IGF-1, insulin-like growth factor; IL, interleukin; MCL1, myeloid leukemia cell differentiation protein 1; MIP, macrophage inflammatory protein; mTOR, mammalian target of rapamycin; PKC, protein kinase C; SDF1 $\alpha$ , stromal cell-derived factor 1 $\alpha$ ; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VLA, very-late antigen.

and reduce osteoclast activity in myeloma. Macrophage inflammatory protein (MIP) 1 $\alpha$ , or chemokine C-C motif ligand, is also produced by myeloma cells and promotes maturation of precursor cells into osteoclasts<sup>190–192</sup>; MIP-1 $\alpha$  signals via CCR1 and CCR5 on osteoclasts and can further upregulate RANKL in stromal cells.<sup>190</sup> MIP-1 $\alpha$  levels are elevated in myeloma patients,<sup>193,194</sup> whereas MIP-1 $\alpha$  silencing or blockade of CCR1 reduces bone disease in *in vitro* or animal models. IL-6,<sup>195</sup> parathyroid hormone–related peptide (PTHrP),<sup>196,197</sup> annexin II,<sup>198</sup> and the ephrinB2/EphB4 axis<sup>199</sup> also promote bone reabsorption. Osteoblast suppression is another major player in myeloma bone disease: WNT signaling antagonists, including DKK1, frizzled related protein-2 (FRP-2),<sup>200</sup> and sclerostin (SOST)<sup>201,202</sup> interfere with osteoblast maturation. DKK1 is expressed by myeloma cells and can upregulate RANKL levels in osteoblasts, increasing osteoclast activity.<sup>203–205</sup> DKK1 levels are increased in the serum of myeloma patients,<sup>206</sup> and anti-DKK1 antibodies have been tested in animal studies<sup>207–210</sup> and are currently employed in clinical trials. Finally, high levels of Activin A, a member

of the transforming growth factor-beta (TGF- $\beta$ ) superfamily; IL-3; and IL-7 (via runt-related transcription factor 2/core-binding factor subunit alpha-1 [RUNX2/CBFA1] blockade) can inhibit bone formation and promote bone reabsorption.<sup>211–213</sup> Furthermore, the bone niche itself supports myeloma cell survival and prevents TNF- $\alpha$ -mediated apoptosis by producing various molecules.<sup>167</sup> Indeed, bisphosphonates not only block osteoclasts and modulate osteoblasts but also have an effect on tumor burden.<sup>214</sup> A similar effect is reported with OPG peptidomimetics and RANKL constructs in *in vivo* xenograft models.<sup>215</sup> Hence, bisphosphonates, especially zoledronic acid, are currently used in the clinic to reduce bone disease,<sup>216,217</sup> but are also associated with an increase in OS when compared to placebo based on a meta-analysis.<sup>218</sup> Markers of bone resorption and formation correlate with the extent of osteolytic disease.<sup>219</sup> Specifically, urine levels of pyridinoline (PYD) and deoxypyridinoline (DPD) cross-links and serum levels of tartrate-resistant acid phosphatase isoform 5b (TRACP-5b), a resorption marker only produced by activated osteoclasts and of collagen degradation

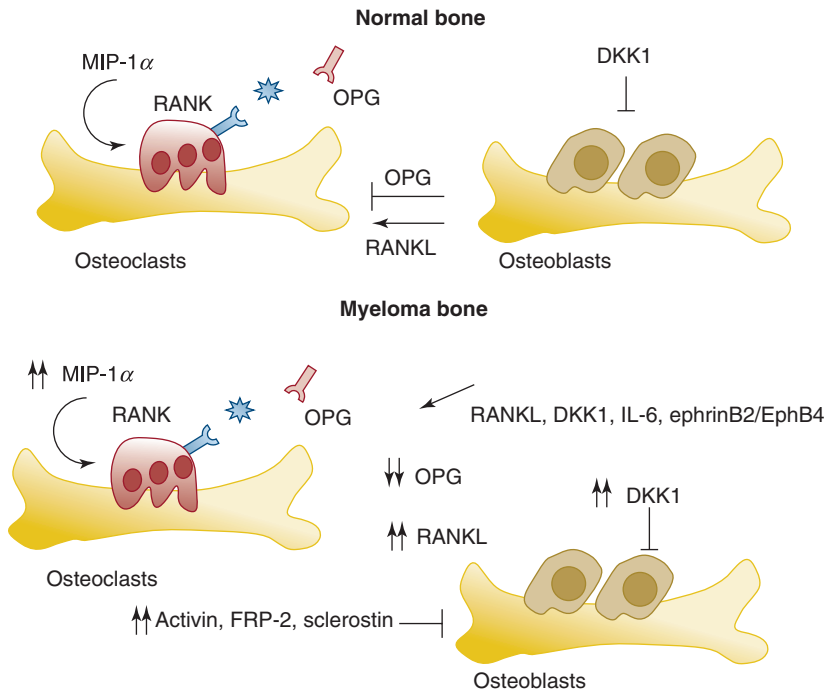


**Figure 18-4.** Mechanism of bone remodeling in normal conditions and in the presence of myeloma cells. The major factors affecting osteoclast and osteoblast activation, and thus the balance between bone formation and bone reabsorption, are illustrated in the upper panel. Receptor activator of nuclear factor- $\kappa$ B (RANK) receptor/receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) and macrophage inflammatory protein (MIP) 1 $\alpha$  stimulate osteoclastogenesis and osteoclast activity, while osteoprotegerin (OPG) acts as a decoy receptor for RANKL, reducing its action. DKK1 (Dickkopf-1) is an inhibitor of osteoblast activity. In the presence of myeloma cells in the bone, the normal balance between osteoblasts and osteoclasts is totally inverted. Specifically, myeloma cells secrete factors to promote osteoclast activation, a result of upregulation of RANKL and MIP-1 $\alpha$ , and to inhibit osteoblasts. Increased levels of DKK1, activin, FRP-2 (frizzled-related protein 2), and sclerostin are evident in myeloma patients. In red are marked cytokines or receptors used as targets to treat myeloma bone disease. IL, interleukin.

products, including the N-terminal cross-linking telopeptide of type I collagen (NTX), are elevated in myeloma patients compared with healthy controls and can predict early progression of bone disease in myeloma. Conversely, bone formation markers, such as bone alkaline phosphatase (bALP) and osteocalcin (OC), are reduced.<sup>219</sup>

## PRECLINICAL MODELS OF MYELOMA

Novel therapies need to be tested in preclinical *in vitro/vivo* models capable of mimicking the role of human marrow microenvironment. In *in vitro* settings, myeloma cells are cocultured in liquid or semisolid systems together with different cytokines (IL-6, IGF-1, TNF- $\alpha$ ) or with autologous BMSCs from patients. However, these systems do not truly

recapitulate the marrow microenvironment,<sup>220–222</sup> and *in vivo* models are necessary. Two main types of myeloma animal models have been exploited to study human myeloma biology and response to treatment: xenogeneic models in immunodeficient and humanized mice or syngeneic tumor models (Fig. 18-5).<sup>223</sup> The xenogeneic models require subcutaneous injection of tumor myeloma cells in severe combined immune deficiency (SCID) or nonobese diabetic (NOD)/SCID mice, which are immunocompromised. These models lack the complex marrow-myeloma interaction but can still be used to explore myeloma homing and novel drugs. Conversely, the SCID-hu or the SCID-rab mice models re-create a look-alike microenvironment able to sustain myeloma cell growth.<sup>224</sup> Specifically, primary myeloma cells or myeloma cell lines are grown in human fetal bone (the SCID-hu model) or rabbit bone

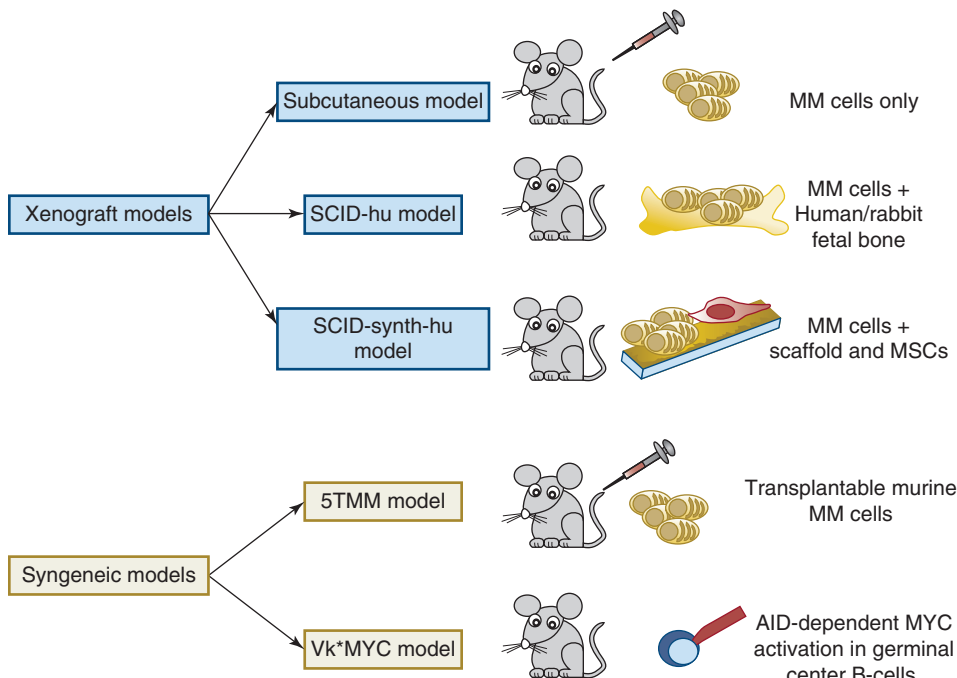


**Figure 18-5.** Preclinical models of myeloma. Xenogeneic and syngeneic models have been developed to study myeloma biology and test novel therapies. In xenogeneic models, human myeloma (MM) cells are injected subcutaneously, inside human/rabbit fetal bone, or into synthetic scaffolds, previously coated with mesenchymal stromal cells (MSCs). In the last two types of models, a look-alike microenvironment, able to sustain myeloma cell growth, is present. Two types of syngeneic models have been established: The first one consists of the transplant of murine myeloma cells into other mice (5TMM), while the second one (Vk\*MYC mouse) is a genetically engineered mouse where MYC is activated in germinal center B cells via an activation-induced cytidine deaminase (AID)-dependent mechanism. SCID, severe combined immune deficiency.

(SCID-rab model), later implanted in SCID mice. Myeloma cells grow inside the implanted bones or disseminate to the outer surface of the implanted bone if they derive from patients with extramedullary disease (EMD). Moreover, these mice have circulating monoclonal immunoglobulins, osteolytic lesions, suppression of bone formation near myeloma foci, and angiogenesis.<sup>224,225</sup> Studies propose novel xenogeneic models, where three-dimensional bone-like scaffolds are used instead of human fetal bone or rabbit bone.<sup>226,227</sup> Specifically, these scaffolds are internally coated with murine/human BMSCs or human mesenchymal stromal cells and then implanted in SCID or RAG2-/- $\gamma$ c-/- mice to recapitulate the autologous marrow microenvironment *in vivo*. Myeloma cells are then loaded directly inside the scaffold or injected intracardiac to mimic myeloma homing. These models are more suitable for studying the microenvironment and also for drug testing, affording the opportunity to evaluate different stages or type of disease. Syngeneic tumor models include transplantable murine models like 5T33,<sup>228,229</sup> which are mostly representative of aggressive late-stage disease, as well as genetically engineered mouse models, such as the Vk\*MYC mouse,<sup>230</sup> generated from the C57BL/6 mouse strain, which has a high incidence of spontaneous monoclonal gammopathy with aging, after activation-induced cytidine deaminase-dependent MYC activation in germinal center B-cells. This model exhibits high penetrance; clonal malignant plasma cells, which produce isotype class-switched immunoglobulins; a similar histology and immunophenotype to human myeloma; and delayed onset of renal failure, bone lesions, and anemia. The Vk\*MYC mouse model has been used for drug testing, strongly paralleling the drug activity observed in myeloma patients.<sup>230</sup>

## CLINICAL AND LABORATORY FEATURES

Table 18-1 summarizes the signs and symptoms associated with myeloma. The International Myeloma Working Group (IMWG) has issued simplified criteria for the classification of myeloma and related disorders.<sup>231,232</sup> Symptomatic myeloma is diagnosed by evidence of organ or tissue impairment (end-organ damage) manifested by anemia, hypercalcemia, lytic bone lesions, renal insufficiency, hyperviscosity, amyloidosis, or recurrent infections (commonly referred to as the “CRAB” criteria, namely, hypercalcemia, renal failure, anemia, and bone lesions) (Tables 18-2 and 18-3; Fig. 18-6). Presence of these features necessitate immediate treatment for myeloma.

## HEMATOLOGIC ABNORMALITIES

Myelomatous involvement of the marrow typically causes anemia, which is present in more than two-thirds of patients with myeloma and relates to the degree of marrow infiltration. The erythropoietin response is insufficient in myeloma, owing to production of cytokines (IL-1, TNF- $\beta$ , Fas ligand, MIP-1 $\alpha$ , and tumor necrosis factor-related apoptosis-inducing ligand [TRAIL], which produce erythroblast apoptosis)<sup>233</sup>, increased serum viscosity, or concomitant renal dysfunction.<sup>234-236</sup> Patients with myeloma have high urinary and serum hepcidin levels that inversely correlate with hemoglobin values.<sup>237,238</sup> IL-6 and bone morphogenetic protein (BMP) 2 mediate hepcidin transcription in the liver via STAT3 signaling,<sup>239</sup> which in turn blocks iron release from macrophages and inhibits iron absorption from the intestine.<sup>240,241</sup> In contrast to other lymphoproliferative disorders, thrombocytopenia is uncommon at diagnosis, even with extensive marrow infiltration, as IL-6 has thrombopoietic activity.<sup>242</sup> In some patients, thrombocytopenia might occur secondary to treatment or to autoimmune mechanisms (such as those accounting for anemia or factor VIII deficiency<sup>46,243-245</sup>). Specifically, bortezomib causes a cyclic thrombocytopenia, with a different kinetic

**TABLE 18-2.** Criteria for Diagnosis of Myeloma

1. Clonal bone marrow plasma cells  $\geq 10\%$  of biopsy-proven bony or extramedullary plasmacytoma
2. Any one or more of the following myeloma-defining events:
  - Evidence of end-organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
    - Hypercalcemia: serum calcium  $>0.25$  mmol/L ( $>1$  mg/dL) higher than the upper limit of normal or  $>2.75$  mmol/L ( $>11$  mg/dL)
    - Renal insufficiency: creatinine clearance  $<40$  mL per min or serum creatinine  $>177$   $\mu$ mol/L ( $>2$  mg/dL)
    - Anemia: hemoglobin value of  $>20$  g/L below the lower limit of normal or a hemoglobin value  $<100$  g/L
    - Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT
  - Any one or more of the following biomarkers of malignancy:
    - Clonal bone marrow plasma cell percentage  $\geq 60\%$
    - Involved:uninvolved serum free light-chain ratio  $\geq 100$
    - $>1$  focal lesion on MRI studies

CT, computed tomography; PET, positron emission tomography.

Modified with permission from Rajkumar SV, Dimopoulos MA, Palumbo A, et al: International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma, *Lancet Oncol* 2014 Nov;15(12):e538-e54

**TABLE 18-3.** Symptomatic Myeloma<sup>247</sup>

| Symptoms and Laboratory Features                                      | Frequency (%)                              |
|-----------------------------------------------------------------------|--------------------------------------------|
| Bone pain (spine, chest, less common in long bones)                   | 58                                         |
| Weakness and fatigue                                                  | 32                                         |
| Anemia                                                                | 73                                         |
| Elevated creatinine                                                   | 48                                         |
| Hypercalcemia                                                         | 28                                         |
| Serum monoclonal immunoglobulin (Ig) peak on standard electrophoresis | 82                                         |
| Weight loss                                                           | 24 (one-half of whom had lost $\geq 9$ kg) |
| Monoclonal Ig peak on immunofixation of serum or urine                | 97                                         |
| Monoclonal IgG                                                        | 52                                         |
| Monoclonal IgA                                                        | 21                                         |
| Monoclonal light chains only                                          | 16                                         |
| Monoclonal IgD                                                        | 2                                          |
| Biclonal                                                              | 2                                          |
| Monoclonal IgM                                                        | 0.5                                        |
| Negative                                                              | 6.5                                        |
| Urinary monoclonal light chains                                       | 75                                         |
| Marrow plasmacytosis $>10\%$                                          | 90                                         |

Data from Kyle RA, Gertz MA, Witzig TE, et al: Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc* 2003 Jan;78(1):21-33.



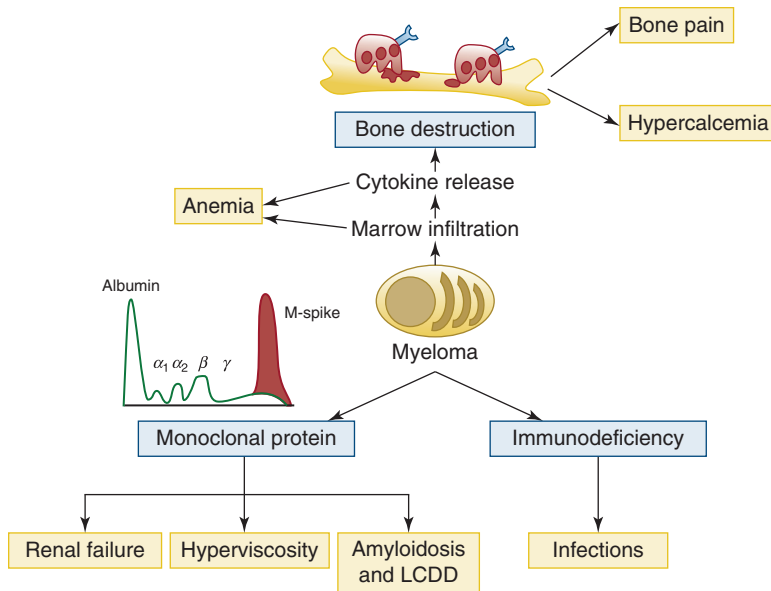


**Figure 18-6.** Summary of clinical manifestations of myeloma. Bone destruction, immunodeficiency, and presence of a monoclonal protein account for the main factors capable of inducing symptoms in myeloma patients. Anemia, hypercalcemia, bone pain, and renal failure represent the classical symptoms of myeloma presentation, together with increased risk of infections. Hyperviscosity and amyloid light-chain amyloidosis or light-chain deposition disease (LCDD) are less common presenting situations.

from cytotoxic drugs, appearing during the first 10 days of each cycle, but with a short recovery time, no cumulative or persistent effects, and absence of marrow megakaryocyte toxic damage, as it primarily results from a functional alteration in platelet budding.<sup>246</sup> Prolonged exposure to alkylating agents can also promote onset of a concomitant myelodysplastic syndrome. Overt bleeding is a relatively uncommon presenting symptom for myeloma patients<sup>247</sup>; however, it occurs more commonly with IgA paraproteins, in the presence of very high concentrations of serum immunoglobulins, high serum viscosity, and a prolonged bleeding time, with normal platelet counts, prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time.<sup>248,249</sup> Acquired von Willebrand factor (VWF) deficiency can develop<sup>250</sup> as a result of plasma VWF-neutralizing antibodies, antibodies binding to the VWF glycoprotein 1b binding domain or interfering with VWF binding to collagen,<sup>251,252</sup> or immunoglobulins that nonspecifically coat platelets or bind to fibrin, preventing aggregation. An asymptomatic prolonged thrombin time can also be present<sup>253</sup> as a result of interference with fibrin clot formation by the monoclonal protein<sup>254</sup>; in a few cases, paraproteins recognizing thrombin and factor VIII have been reported.<sup>253,255</sup> Bleeding may also result from progression of disease, renal insufficiency, infections, therapy-related toxicity, invasive procedures, and anoxia/thrombosis in the capillary circulation. Bleeding is more common in systemic AL amyloidosis (15 to 41 percent of patients at diagnosis)<sup>256–258</sup> because of the deposition of free immunoglobulin light chains forming insoluble fibrils in the small vessels or, more rarely, as a result of acquired factor X deficiency, related to absorption of factor X onto AL fibrils in the liver and spleen.<sup>259,260</sup> Amyloid splenic infiltration can cause hyposplenism and thrombocytosis.<sup>260</sup> An increased risk of venous thromboembolic events is typical of MG and myeloma patients.<sup>261,262</sup> Hypercoagulable states may result from the proinflammatory activity of IL-6; abnormal interactions between myeloma cells, BMSCs, and endothelial cells<sup>263</sup>; paraprotein effects on fibrin polymerization and resistance to fibrinolysis; or rarely neutralizing antibodies against protein C, S,<sup>264,265</sup> or lupus anticoagulant<sup>266</sup> and acquired protein C resistance.<sup>267</sup> Immunomodulatory drugs (IMiDs), such as thalidomide and lenalidomide, have antiangiogenic properties and are associated with an increased risk of venous thromboembolism (VTE), ranging from 5 percent<sup>268</sup> to 18 percent of treated patients,<sup>269</sup> especially when combined with high-dose dexamethasone, doxorubicin, or erythropoietic agents.<sup>270–274</sup> Warfarin and low-molecular-weight heparin (LMWH) play a role in primary and

secondary VTE prevention,<sup>275</sup> but aspirin is normally used in patients treated with IMiDs and steroids in view of the presence of increased aggregation between platelet and VWF antigens.<sup>276–279</sup>

## IMMUNOGLOBULIN ABNORMALITIES

The majority of myeloma patients produce and secrete a monoclonal immunoglobulin (M protein or M spike) that can be detected by protein electrophoresis of the serum (SPEP) and/or of urine (UPEP) after a 24-hour urine collection. The M protein presents as a single narrow peak, migrating in the  $\gamma$ , or rarely  $\beta$ , region of the densitometer tracing. Myeloma cells can secrete immunoglobulin heavy chains plus light chains, light chains alone, or neither (nonsecretory myeloma). In this last case, cytoplasmic immunoglobulins are detected. Immunofixation analysis identifies the unique and specific immunoglobulin idiotypes.<sup>47</sup> Monoclonal IgG (usually  $>3.5$  g/dL) is present in approximately 60 percent of myeloma patients, monoclonal IgA (typically  $>2$  g/dL) in 20 percent of patients, monoclonal immunoglobulin light chains alone are detected in 20 percent of patients, while IgD, IgM, and biclonal myeloma are rare (5 percent of cases). A low M-spike concentration is particularly suggestive of IgD myeloma isotype. Light-chain myeloma patients should be followed by UPEP and urine immunofixation and present more often with renal failure or increased creatinine levels. Light-chain proteinuria is frequent especially in IgD myeloma (Table 18-3). Traditionally, IgA and especially IgD isotypes have been considered prognostically unfavorable.<sup>280,281</sup> Their prognostic value has been confirmed in patients enrolled on Total Therapy 1, 2, and 3 protocols; IgD was of borderline significance, mainly because of its rarity, but was strongly associated with elevated  $\beta_2$ -microglobulin ( $\beta_2M$ ) and lactic dehydrogenase (LDH) serum levels, reflecting high tumor burden.<sup>282</sup> Suppression of normal, polyclonal serum immunoglobulins resulting in total hypoglobulinemia and an increased risk of infection is present in 70 to 90 percent of patients. The  $\kappa$  light-chain isotype is twice as common as the  $\lambda$  isotype, except in IgD myeloma. The free light-chain (FLC) assay (FREELITE assay) is a novel technique used to detect monoclonal FLCs and provides an FLC  $\kappa:\lambda$  ratio (Fig. 18-7).<sup>283</sup> The  $\kappa:\lambda$  ratio is considered abnormal if less than 0.26 ( $\lambda$ -restricted immunoglobulin) or more than 1.65 ( $\kappa$ -restricted immunoglobulin).<sup>284</sup> Because the half-life of FLCs is only 2 to 4 hours, in contrast to the half-life of the entire immunoglobulin, which is 17 to 21 days, the FLC assay can be used to detect early treatment responses

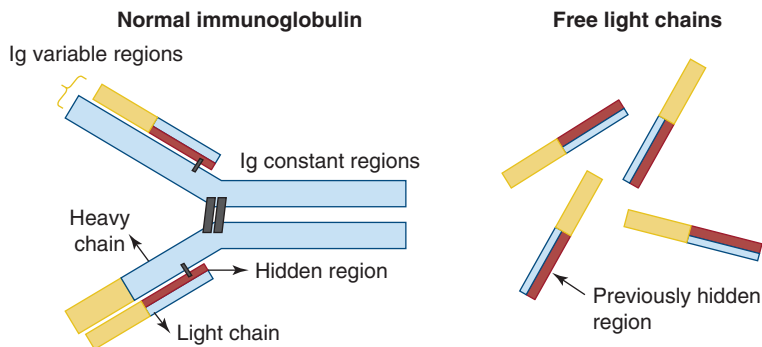


**Figure 18-7.** Free light-chain assay description. Normal immunoglobulins are composed of two heavy chains and two light chains, which together form a constant region and a variable region, capable of recognizing specific antigens. The free light-chain assay is used to quantify the amount of free light chains in myeloma patients and to specifically recognize a “hidden” antigenic region (in red) that is normally not detectable from intact immunoglobulins. Ig, immunoglobulin.

and should be evaluated routinely in patients with AL amyloidosis and oligosecretory myeloma.<sup>285</sup> A normal FLC ratio is also included in the criteria for stringent complete response (sCR).<sup>8</sup> The FLC ratio is considered a predictor of the risk of progression from MG or smoldering myeloma to active myeloma,<sup>286,287</sup> and it has prognostic value, being related to tumor burden.<sup>285</sup> Indeed, high baseline FLC correlates with shorter survival in newly diagnosed myeloma patients despite achievement of complete response (CR).<sup>288,289</sup> A rapid reduction in FLCs after therapy is also linked to inferior OS and event-free survival (EFS), suggesting the presence of highly proliferative myeloma cells, particularly sensitive to combination chemotherapy.<sup>290</sup>

## MARROW FINDINGS

A marrow aspirate or biopsy showing plasmacytosis is a key component for the diagnosis of myeloma. The marrow can be evenly infiltrated (diffuse involvement) but more commonly displays considerable site-to-site variability (focal involvement).<sup>291</sup> The percentage of plasma cells can range from 10 percent to a virtual complete replacement of marrow.

Occasionally, the biopsy specimen may contain a normal proportion of plasma cells as a result of the patchy marrow involvement. Myeloma diagnosis is also considered in the presence of less than 10 percent of chain-restricted plasma cells if symptoms are reported or after histopathologic confirmation of an intraosseous or extraosseous plasmacytoma. By light microscopy, the morphologic appearance of myeloma plasma cells can be indistinguishable from normal plasma cells, characterized by abundant basophilic cytoplasm and round, eccentrically located nuclei, with “clock-face” or “spoke-wheel” chromatin without nucleoli or by bizarre plasma cells with giant cellular size, open chromatin, and punched nucleoli (indicating increased transcriptional activity); by a high frequency of binucleate or multinucleate cells; and by the presence of inclusion globules of condensed or crystallized cytoplasmic immunoglobulin, including Russell bodies (cherry-red refractive round bodies), multiple pale bluish-white, grape-like accumulations (Mott cells or Morula cells), crystalline rods, glycogen-rich IgA (flame cells), or other inclusions. These abnormal cells are characteristic of plasmablastic myeloma,<sup>292,293</sup> a poor prognostic type of myeloma with a high number of mitotic figures (Figs. 18-8 to 18-10).<sup>294</sup> Myeloma cells are clonal by

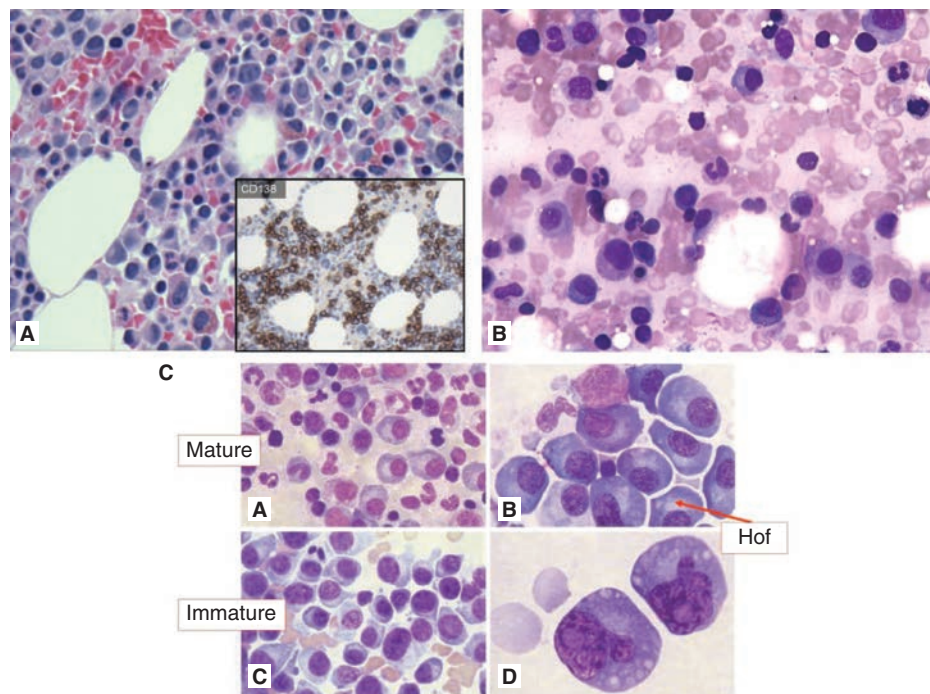


**Figure 18-8.** Marrow findings in myeloma. **A.** Marrow film. Infiltrate of neoplastic plasma cells (myeloma cells). These cells resemble normal plasma cells in their appearance with characteristic nuclear:cytoplasmic area ratio, blocky nuclear chromatin pattern, intense cytoplasmic basophilia, and a prominent “hof” or clear area (Golgi zone). CD138 immunohistochemistry staining is shown in the small box. **B.** Marrow film. Infiltrate of malignant plasma (myeloma) cells. **C(A).** Marrow section. Mature malignant plasma cells. **C(B).** Marrow film. Malignant plasma cells showing prominent perinuclear Golgi apparatus (Hof). **C(C)** and **C(D).** Immature plasma cells with abnormal nuclei and size and aberrant morphology.



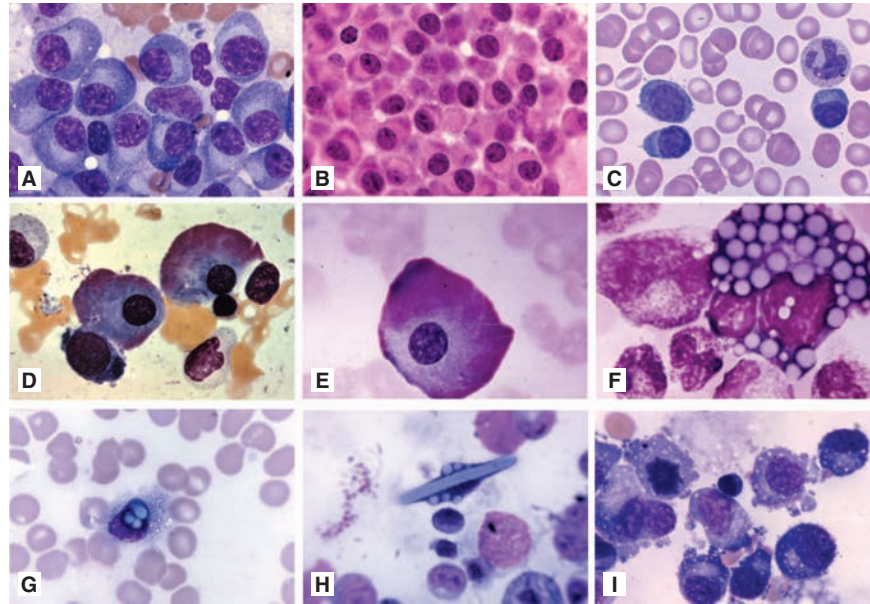


**Figure 18-9.** Myeloma: morphologic appearances. **A.** Marrow film showing replacement by malignant plasma (myeloma) cells. Note classical oval cell shape, eccentric nucleus, striking paranuclear clear area, deeply blue cytoplasm. **B.** Marrow biopsy section showing replacement by myeloma cells. **C.** Blood film in a patient with plasma cell leukemia. Three myeloma cells in the blood film field. **D** and **E.** Marrow films. Flaming-type giant myeloma cells. Reddish peripheral cytoplasmic coloration reflecting very high concentration of carbohydrate, characteristic of immunoglobulin (Ig) A myeloma. The peripheral cytoplasm contains numerous dilated cisterns of the endoplasmic reticulum distended with immunoglobulin. Flaming plasma cells may occasionally be found in IgG myeloma and in reactive plasmacytosis. **F.** Morula or Mott cell. Myeloma cell engorged with globules presumably containing immunoglobulin. These globules individually are referred to as Russell bodies, and plasma cells may be found containing one, several, or many such bodies. **G.** Plasma cell with immunoglobulins containing globules overlying the nucleus but presumably cytoplasmic in location along with smaller cytoplasmic globular inclusions. **H.** Immunoglobulin crystal with several globules of immunoglobulin on either side. Note remarkable distortion of the cell to accommodate the crystal. **I.** Marrow film. Myeloma cells exhibiting cytoplasmic shedding. (Reproduced with permission from Lichtman's Atlas of Hematology, [www.accesmedicine.com](http://www.accesmedicine.com).)

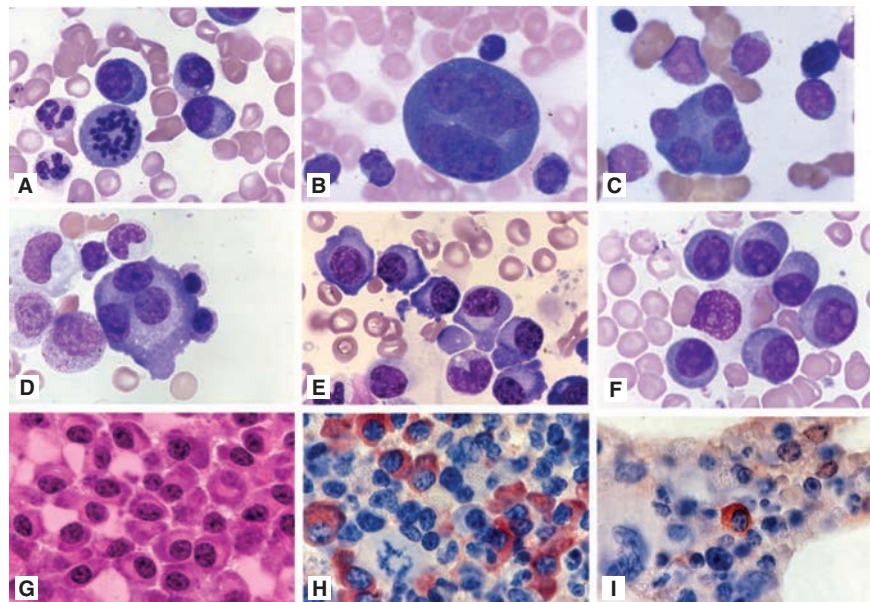


**Figure 18-10.** Myeloma: Additional morphologic appearances. **A.** Marrow film. Three characteristic malignant plasma (myeloma) cells and one in mitosis. **B.** Marrow film. Giant multinucleated myeloma cell. **C.** Marrow film. Tetranucleated myeloma cell. **D.** Marrow film. Trinucleated myeloma cell. **E.** Marrow film. Infiltrate of classical well-differentiated myeloma cells (plasma cell phenocopies). Eccentric nuclei, paranuclear clear zone, deeply blue (basophilic) peripheral cytoplasm, less-discrete paranuclear clear zone (plasmablasts). **F.** Infiltrate of immature myeloma cells with more circular than ovoid shapes, very large, prominent large nucleoli, less-intense basophilic cytoplasm, less-discrete paranuclear clear zone (plasmablasts). **G** to **I.** Marrow biopsy sections showing striking infiltrate of myeloma cells. **G.** Hematoxylin-and-eosin stain. **H.** Immunostained for  $\kappa$  light chains, showing frequently positive cells evident by deep rust color in cytoplasm. **I.** Immunostained for  $\lambda$  light chains showing negative reaction with rare positive cell. Approximately 20:1  $\kappa$ : $\lambda$  ratio. (Reproduced with permission from Lichtman's Atlas of Hematology, [www.accesmedicine.com](http://www.accesmedicine.com).)



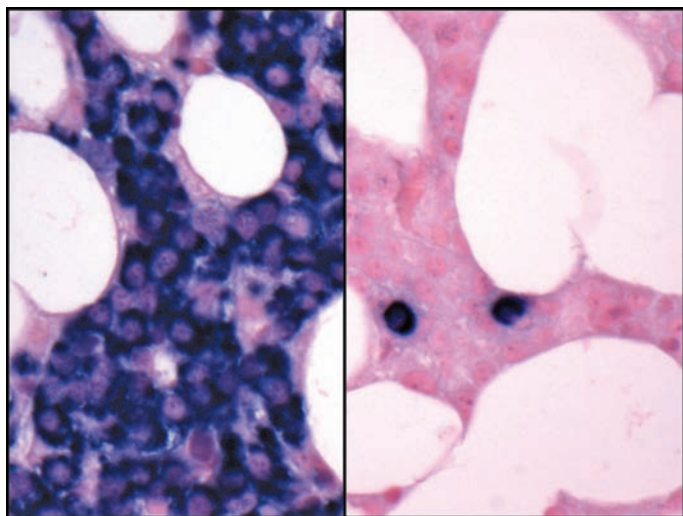


**Figure 18-11.** Kappa-lambda staining.

definition and produce either  $\kappa$  or  $\lambda$  light chains, which are present in the cytoplasm but not on the membrane surface. A  $\kappa$ : $\lambda$  ratio greater than 4:1 (2:1 is normal<sup>295</sup>) or less than 1:2 is considered an index of  $\kappa$  or  $\lambda$  monoclonality, distinguishing this condition from reactive plasmacytosis.<sup>296</sup> Two-parameter flow cytometry staining for nuclear DNA content by propidium iodide and anti- $\kappa$  and anti- $\lambda$  light chains can also be used to quantitate marrow involvement (Fig. 18-11).<sup>297</sup> Myeloma cells are normally CD138+, CD45-, CD38+, and CD19-<sup>298</sup> and are CD56+ in 70 percent of patients.<sup>299-301</sup> A few cases are CD20+<sup>302</sup> or CD117+ (KIT),<sup>303</sup> but responses to treatment with rituximab or imatinib mesylate are uncommon. If amyloid deposition is suspected, Congo red staining can be performed on the marrow biopsy, showing diffuse involvement or focal perivascular niche localization of amyloid protein. MVD can be assessed by staining for endothelial markers such as CD131 and CD34 in specialized laboratories or during clinical trials.<sup>304</sup> Secondary myelodysplastic changes can rarely develop after prolonged treatment in myeloma patients, presenting with pancytopenia in the context of a hypercellular marrow, with characteristic FISH abnormalities.<sup>305,306</sup> Metaphase cytogenetic studies and interphase FISH analysis should be performed routinely on myeloma cells at diagnosis to evaluate the presence of abnormal karyotypes and poor prognostic chromosomal abnormalities, such as deletion of chromosome 17, gain of chromosome 1q, loss of chromosome 1p, deletion of chromosome 13, and t(4;14) or t(14;16) translocations (Figs. 18-12 and 18-13).<sup>63,307-310</sup> Genetic analysis should be repeated at relapse only in patients initially classified as genetic standard risk to rule out emergence of a more aggressive clone.<sup>311</sup> The plasma cell labeling index (LI) corresponds to the percentage of plasma cells in the S phase of the cell cycle and is measured by immunofluorescence staining using an antibody against 5-bromo-2'-deoxyuridine, which is actively incorporated by DNA on marrow plasma cells. Actively cycling myeloma cells represent a small proportion of the total malignant cells, normally 0.5 percent on average,<sup>312-317</sup> with few patients with a LI of more than 5 percent.<sup>318,319</sup> This value has been proposed as a myeloma prognostic marker, as patients with a LI of more than 0.5 percent at diagnosis have a shorter EFS and OS.<sup>318</sup>

## RENAL DISEASE

Increased creatinine levels (>1.5 to 2.0 mg/dL) occur in 30 to 50 percent of myeloma patients at diagnosis, while overt renal failure requiring hemodialysis affects up to 10 percent of patients.<sup>247</sup> Renal

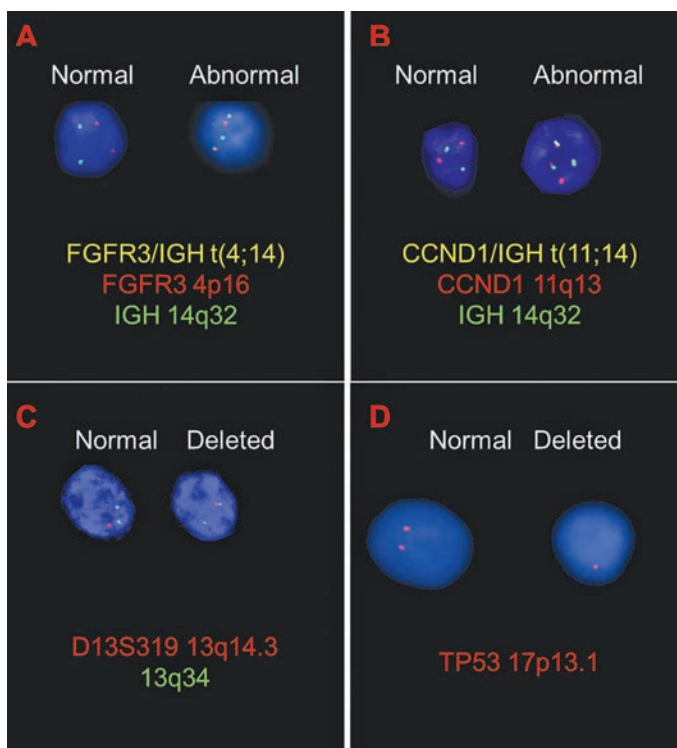


**Figure 18-12.** Common fluorescence *in situ* hybridization (FISH) abnormalities in myeloma. **A.** t(4;14). **B.** t(11;14). **C.** Deletion 13. **D.** Deletion 17p. IGH, immunoglobulin heavy chain.

insufficiency is related to two major causes: myeloma cast nephropathy (also called light-chain cast nephropathy or myeloma kidney) and hypercalcemia.<sup>320</sup> In myeloma cast nephropathy, the tubular absorptive capacity for light chains is overwhelmed, leading to the formation in the distal convoluted tubule (DCT) of the nephron of tubular casts. These tubular casts derive from the binding of precipitated light chains to Tamm-Horsfall mucoprotein (uromodulin) and can obstruct the DCT and parts of the ascending loop of Henle, initiating a giant cell reaction that leads to interstitial inflammation and fibrosis (interstitial nephritis).<sup>321</sup> The cast formation rate is strongly related to the urinary free light-chain concentration, which can be estimated by the amount of total proteinuria, based on the 24-hour urine collection or the serum light-chain values. Conversely, the urine dipstick may be negative for protein as immunoglobulin light chains are often not detected by this technique. Lambda light chains tend to be more nephrotoxic than the  $\kappa$  type, and renal impairment can be present with minimal  $\lambda$  light-chain secretion. Hypercalcemia (calcium >11 mg/dL), the second cause of nephropathy, is present in 15 percent of patients at diagnosis. Hypercalcemia creates volume depletion, natriuresis, and renal vasoconstriction, with an increased risk of prerenal azotemia; moreover, it can lead to intratubular calcium deposition, increasing the toxicity of filtered light chains or cause a reversible form of nephrogenic diabetes insipidus.<sup>322</sup> Light-chain glomerulopathy is caused by the deposition of immunoglobulins in the form of either amyloid or nonamyloid. In AL amyloidosis, light-chain immunoglobulin proteinuria is associated with glomerular damage, resulting in an overt nephrotic syndrome (Chap. 19).<sup>323</sup> Light chains are converted into insoluble fibrils or granular deposits inside the mesangial cells, causing Congo red-positive amyloid accumulation, localized predominantly in the glomeruli. Vascular and tubular amyloid deposits are less common but can cause narrowing of the vascular lumens or tubular dysfunction, such as type 1

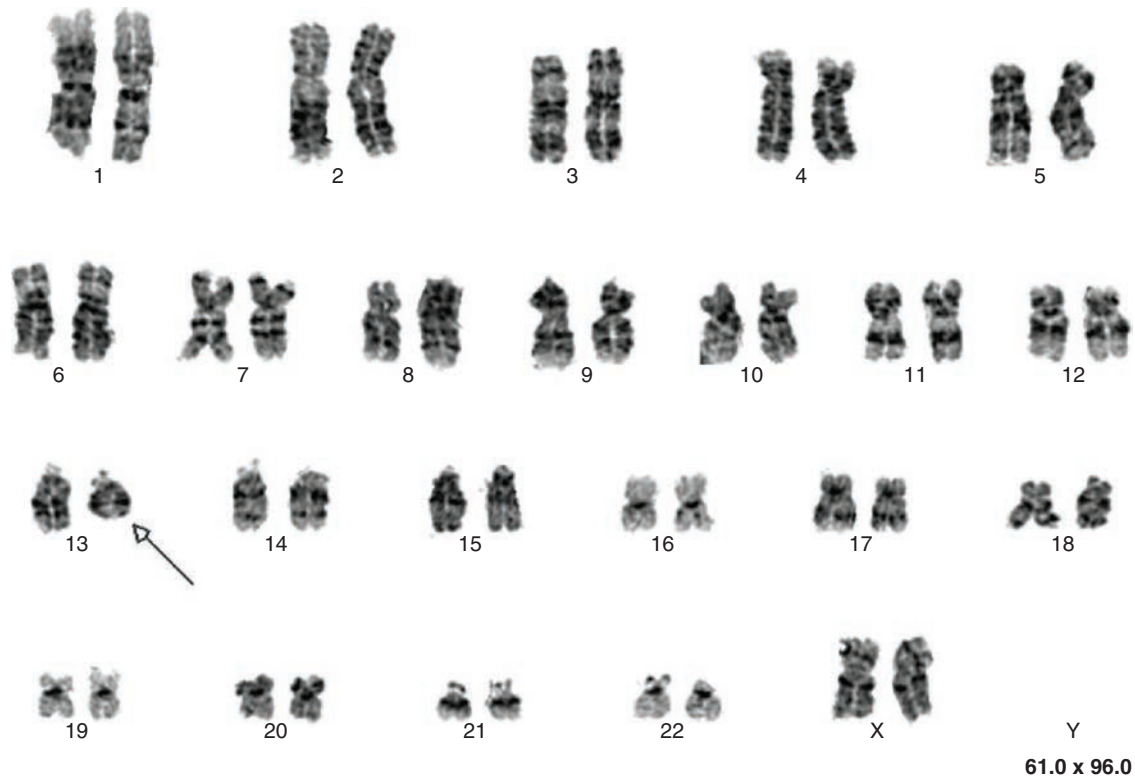


**Figure 18-13.** Abnormal karyotype in myeloma. Deletion of del(13)(q14q31).

(distal) renal tubular acidosis or nephrogenic diabetes insipidus.<sup>324</sup> AL amyloidosis with renal dysfunction is more common in patients with  $\lambda$  light chains, especially those with the  $\lambda$  VI light-chain subgroup.<sup>325</sup> Kappa chains or heavy-chain fragments can form Congo red–negative nonfibrillar deposits with linear involvement of the basement membrane, in a condition called LCDD or, more generally, monoclonal immunoglobulin deposition disease (MIDD). The clinical presentation of LCDD is heralded by development of the nephrotic syndrome followed by renal failure or acquired Fanconi syndrome (more often associated with  $\kappa$  light-chain deposition). In these deposition diseases, the urine dipstick for protein is positive as a result of the glomerular leakage of albumin.<sup>256,324</sup> Renal enlargement can be caused by AL amyloidosis (Chap. 19) or, less commonly, by renal plasmacytomas.<sup>326</sup> Renal vein thrombosis; hyperviscosity; dehydration; use of nephrotoxic drugs (antibiotics, nonsteroidal antiinflammatory drugs, imaging contrast agents—especially when rapidly infused)<sup>327</sup>; hyperuricemia; and type I cryoglobulinemia can all induce or aggravate renal impairment in myeloma patients. Bisphosphonates, in particular, should be infused slowly, at adjusted doses, based on creatinine values. Renal biopsy is usually unnecessary unless nephrotic syndrome is present. However, if systemic amyloidosis or, less likely, LCDD is suspected, a subcutaneous fat aspirate or rectal biopsy should be performed first and tested for amyloid deposits.<sup>320</sup> Renal biopsy specimens should be processed fresh-frozen to allow for immunofixation studies, including electron microscopy and Congo red staining for amyloidosis. Supportive care associated with prompt initiation of antimyeloma treatment is the cornerstone of the management of renal impairment in myeloma. To correct hypercalcemia, aggressive hydration, use of calcitonin, and a slow infusion of one single dose of a bisphosphonate is applied. Cytoreductive chemotherapy should be started as soon as possible. Rapid removal of light chains by plasma exchange is a controversial technique; the introduction of high-cutoff hemodialysis, which employs novel dialysis

filters capable of clearing away free light chains, is showing promising results and improved patient outcomes.<sup>328–330</sup>

In general, myeloma renal impairment is reversible in approximately 50 percent of patients. Conversely, amyloid- and LCDD-related renal impairment tends to be stable or progressive. Patients presenting in acute renal failure have a high early mortality, with up to 30 percent dying within the first months. Improvements in renal function rarely occur after 6 months from diagnosis. Renal dysfunction is a negative prognostic factor, and it results in use of suboptimal therapies, longer hospitalization, and an increased risk of infection. Hence, patients who recover normal kidney function have a better outcome compared to those who do not.<sup>331–333</sup>

## PAIN

Back or chest bone pain as result of vertebral or rib fractures at sites of osteopenia or from lytic bone lesions is present at the time of diagnosis in approximately 60 percent of patients.<sup>247</sup> The pain is usually worse with movement and at night. Pathologic fractures of long bones can ensue as well. Kyphosis or reduction of patient's height is another common feature. Localized pain can also derive from focal plasmacytomas, presenting as expanding masses compressing the spinal cord or nerve roots. Amyloid deposits can provoke painful mass effects when localizing into nerve sheaths, as in amyloid-associated carpal tunnel syndrome.<sup>334</sup>

## INFECTIONS

Myeloma patients are at an increased risk for infections that represent a leading cause of morbidity and mortality. Several aspects contribute to infection risk, including immune dysfunction in the innate and adaptive immune systems<sup>335</sup>; extrinsic factors, like type and duration of therapy (e.g., cytotoxic agents, glucocorticoids, lenalidomide, autologous/allogeneic hematopoietic stem cell transplantation [HSCT]); and physical

factors, such as age, coexisting comorbidities, hypoventilation secondary to pathologic fractures, indwelling vascular catheters and impaired mucosal integrity. A broad immune dysfunction involving B lymphocytes, T lymphocytes, NK cells, and dendritic cells is noted in myeloma patients.<sup>335–337</sup> Specifically, myeloma cells or BMSCs can produce a series of immunologically molecules, such as TGF- $\beta$ , IL-10, and IL-6. TGF- $\beta$  and IL-10 suppress IL-2 autocrine pathways, blocking T-cell antitumoral responses<sup>338–340</sup> and stimulate T-regulatory cell proliferation.<sup>341</sup> Moreover, these cytokines sustain the immature phenotype of dendritic cells, highly specialized antigen-presenting cells, reducing their expression of costimulatory molecules (HLA-DR, CD40, and CD80 antigens) and thereby their antigen stimulatory capacity.<sup>342</sup> IL-6 also inhibits dendritic cell production from CD34+ progenitor cells.<sup>342</sup> Normal CD19+ B lymphocytes at early and late stages are suppressed in myeloma, resulting in hypogammaglobulinemia, inversely correlating with the disease stage.<sup>343</sup> B-cell dysfunction in myeloma can be related to TGF- $\beta$  effects, lack of stimulatory signals from helper T cells, and altered gene expression. Hypogammaglobulinemia is particularly responsible for a myeloma patient's susceptibility to encapsulated organisms, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. T-cell subsets are also abnormal, with inversion of the CD4:CD8 ratio<sup>344</sup> and T-helper type 2 (Th2) cell-type skewing.<sup>345</sup> Moreover, global T-cell receptor (TCR) diversity is reduced, with the presence of oligoclonal expansions of CD4+ and CD8+ T cells, and TCR signaling is compromised<sup>344,346,347</sup>;  $\chi\delta$ T cells and NK cells are also aberrant in myeloma. The presence of decreased B-cell or T-cell counts can negatively impact survival.<sup>348</sup>  $\beta_2$ M is the invariant chain of the major histocompatibility (MHC) class I molecule, which is shed by myeloma cells. Its levels correlate with tumor burden and are important for patient staging using the International Staging System (ISS)<sup>232</sup> (see the "Staging" section).  $\beta_2$ M induces IL-6, IL-8, and IL-10 production and activation of STAT3. However, it also has immunosuppressive functions, by reducing surface expression of CD83, HLA-ABC, costimulatory molecules, and adhesion molecules on dendritic cells, impairing stimulatory dendritic-allopecific T-cell responses by inactivation of the Raf/MEK/ERK cascade and NF- $\kappa$ B in dendritic cells.<sup>349</sup> Vaccines, prophylactic antibiotics or antivirals, and intravenous immunoglobulin (IVIG) are preventive measures apt to decrease infections among myeloma patients. Yearly influenza vaccines and a single pneumococcal vaccine at diagnosis are recommended, as myeloma patients can still mount a suboptimal immunologic response. Antiviral prophylaxis (e.g., acyclovir 400 mg twice daily or valacyclovir 500 mg once daily) is mandatory in patients treated with bortezomib combined with glucocorticoid regimens to prevent herpes zoster. Antibiotic prophylaxis is controversial. A study in which patients were randomized on a 1:1:1 basis to receive a daily quinolone, trimethoprim-sulfamethoxazole, or placebo for the first 2 months of treatment did not show a decreased incidence of serious infection (more severe than grade 3 and/or requiring hospitalization), any infection within the first 2 months of treatment, or any improvement in response rate or OS.<sup>350</sup> However, antibiotic prophylaxis with ciprofloxacin or trimethoprim-sulfamethoxazole might be still beneficial in selected patients, such as those patients with a history of repeated infections, those receiving more intense regimens, or those with persistently low CD4+ counts to prevent *Pneumocystis carinii* pneumonia or other infections. IVIG infusion may be considered in patients with recurrent, serious infections despite antibiotic prophylaxis.

## NEUROPATHY

Local myeloma growth can cause polyneuropathy by spinal cord or peripheral nerve compression, even though polyneuropathy is not a common presenting symptom, unless in the context of perineuronal

or perivascular (vasa nervorum) amyloid deposition.<sup>256</sup> POEMS syndrome, or osteosclerotic myeloma, is an exception, where complete polyneuropathy is practically always present along with organomegaly, endocrinopathy, MG, and skin changes.<sup>351,352</sup> The pathogenesis of POEMS syndrome is largely unknown, but chronic overproduction of proinflammatory cytokines, such as VEGF, plays a major role.

## HYPERVISCOSITY

Less than 10 percent of myeloma patients develop signs of hyperviscosity syndrome, a condition that manifests in 10 to 30 percent of patients with Waldenström macroglobulinemia (Chap. 20), because monoclonal IgMs display a higher intrinsic viscosity than other immunoglobulins.<sup>353–355</sup> Cutaneous or mucosal bleeding is very common in hyperviscosity syndrome, together with blurred vision, headache, vertigo, dizziness, nystagmus, deafness, and ataxia. Circulatory problems, affecting cerebral, pulmonary, and renal circulation can rarely ensue in the presence of high blood viscosity (see "Hyperviscosity Syndrome" in Chap. 20).<sup>356</sup> Relative serum viscosity, but not serum immunoglobulin levels, correlates with onset and extent of clinical symptoms. Based on the specific physicochemical properties of classes and subclasses of immunoglobulins, up to one-quarter of patients with IgA myeloma can have blood hyperviscosity as a result of the tendency of IgA to form dimers or polymers.<sup>357</sup> Patients with IgG<sub>3</sub> subclass myeloma, whose immunoglobulins have higher propensity to aggregate, can also manifest with hyperviscosity syndrome.<sup>358</sup>

## PLASMA CELL LEUKEMIA AND EXTRAMEDULLARY DISEASE

Plasma cell leukemia (PCL) is diagnosed when more than 2000 myeloma cells/ $\mu$ L are present in the blood or plasmacytosis accounts for greater than 20 percent of the differential white cell count. PCL is rarely manifest at the time of primary presentation and more commonly arises from preexisting myeloma as end-stage disease. In this case, tumor cells have become microenvironment independent and not only accumulate in the marrow but also recirculate in the blood (EMD).<sup>359–362</sup> However, low levels of circulating plasma cells can be detected in the majority of myeloma patients. By definition, EMD is the presence of a clonal plasmacytic infiltrate outside the marrow. Specifically, it is now considered EMD only if the infiltrate is present at anatomic sites distant from the bone or adjacent soft tissue, hence excluding cases where soft-tissue masses arise in contiguity with the marrow.<sup>363</sup> Indeed, in true EMD, plasma cells have an immature, plasmablastic morphology and have a high proliferative index. According to different clinical trials, 6.0 to 7.5 percent of patients screened with magnetic resonance imaging (MRI) or positron emission tomography-computed tomography (PET-CT) have EMD at the time of diagnosis.<sup>364,365</sup> Extramedullary masses can localize to several organs, including liver, lymph nodes, spleen, kidneys, breasts, pleura, meninges, and cutaneous sites, and are inevitably associated with elevated levels of LDH<sup>366</sup> and a poor response to treatment.<sup>367–369</sup> Leptomeningeal myelomatosis with abnormal cerebrospinal fluid findings is rare but can manifest at advanced stage.<sup>370,371</sup> EMD cells are often CD56- or low and present t(4;14) and del(17p) more commonly,<sup>372,373</sup> together with TP53 mutations, TP53 nuclear localization,<sup>110,374</sup> or high expression levels of focal adhesion kinase (FAK1).<sup>375</sup> In addition, it has been speculated that high-dose melphalan and modern salvage therapies can artificially increase the incidence of EMD as a result of longer duration of treatment, emergence of dormant cells, and poor penetration of the drugs to sanctuary sites like the central nervous system.<sup>376</sup>



## SPINAL CORD COMPRESSION

Spinal cord compression can result from an extramedullary plasmacytoma or vertebral fracture and present acutely with severe back pain alongside weakness or paresthesias of the lower extremities or bladder/bowel incontinence. It should be considered a medical emergency, evaluated with MRI, and promptly treated with a combination of local radiotherapy, decompressive laminectomy, and systemic chemotherapy to avoid permanent deficits. Specifically, local radiotherapy using less than 30 Gy is potentially curative in the presence of a solitary plasmacytoma; in patients with systemic disease, the DT-PACE regimen, which combines high-dose dexamethasone pulsing as part of the combination with oral dexamethasone, daily thalidomide, and 4 days of continuous-infusion cisplatin, doxorubicin, cyclophosphamide, and etoposide, can provide effective treatment and should be followed by local radiation in the absence of symptom relief and lack of tumor shrinkage. If a singular vertebral collapse is evident without plasmacytoma, decompressive laminectomy is the treatment of choice.

## INITIAL EVALUATION OF THE PATIENT WITH MYELOMA

Minimal evaluation requirements include a complete blood count with differential white cell count; examination of a blood film for the presence of rouleaux and circulating myeloma cells; a comprehensive serum metabolic panel for the detection of hypercalcemia, renal failure, serum  $\beta_2$ M, C-reactive protein (CRP), and elevation of LDH (Table 18-4). Myeloma protein studies should include serum protein electrophoresis to quantitate the serum protein electrophoretic pattern in combination with nephelometric quantitation of immunoglobulin levels, serum-free light-chain assay, and a 24-hour urine collection to quantitate 24-hour total urinary protein and determine, using urine electrophoresis, specific urinary proteins, such as light chains or Bence Jones protein.

Immunofixation of serum and urine is needed for the immunoglobulin heavy- and light-chain isotype determination. Serum-free light-chain assay is of particular use in the monitoring of patients with

a plasma monoclonal immunoglobulin, in the diagnosis and monitoring of patients who would otherwise be considered to have nonsecretory myeloma, and for patients who only have light-chain proteinuria. Marrow aspiration and biopsy should include genetic studies (FISH and cytogenetics) and flow cytometry. Newer modalities such as mutational profiling and gene-expression studies are being undertaken but are not yet standard-of-care tests. Radiographic examination usually comprises a metastatic bone survey to detect vertebral compression fractures, osteopenia, and impending fractures of long bones and pelvis. MRI and PET-CT are more sensitive than the bone survey and better capture early bone disease, the extent of bone disease, and EMD. Both MRI and PET-CT findings have important prognostic implications.<sup>209,364</sup> These tests are now being used more frequently specifically in smoldering multiple myeloma (SMM) patients. Assessment of the heart by echocardiogram and electrocardiogram is useful in the right clinical context to detect cardiac amyloidosis and/or LCDD, and, in selected cases, cardiac MRI may be helpful to demonstrate myocardial infiltration. Measurement of brain natriuretic peptide and N-terminal prohormone B-type natriuretic peptide is useful for screening to detect cardiac dysfunction caused by amyloidosis or LCDD.

## STAGING

Multiple attempts have been made to define clinical and laboratory parameters that have prognostic significance in myeloma.<sup>231,377,378</sup> Of the many staging systems, the Salmon-Durie staging system was historically the most commonly used; however, it has been replaced by newer staging systems, which reflect better myeloma biology.<sup>379</sup> The Salmon-Durie system relates myeloma cell mass to the extent of bony disease, hemoglobin, and calcium levels and the monoclonal immunoglobulin levels in serum and urine (Table 18-5). However, measurement of bone disease by skeletal survey in myeloma is observer dependent and potentially subjective.

The ISS is based on two widely available parameters—serum  $\beta_2$ M and albumin—and recognizes three stages (Table 18-6). Stage I is defined by  $\beta_2$ M less than 3.5 mg/L and albumin 3.5 g or greater/100 mL; stage III is characterized by a  $\beta_2$ M of 5.5 mg or greater/L.<sup>231</sup> The intermediate stage II has features of neither stage I nor stage III.  $\beta_2$ M correlates with tumor mass and impairment in renal function, whereas low albumin reflects the effect of IL-6 produced by the microenvironment of myeloma cells on the liver.<sup>380–382</sup> The different ISS stages were predictive of outcome in an analysis of more than 11,000 patients receiving either standard therapies or melphalan-based high-dose therapy followed by autologous hematopoietic stem cell transplantation (auto-HSCT; Table 18-7). Although predictive of outcome, several shortcomings of the ISS include lack of accounting for cytogenetics and bone disease in patients.

## IMAGING STUDIES

Imaging studies are an essential part of the diagnosis and management of myeloma. The standard of care in the initial staging of newly diagnosed myeloma is a complete skeletal survey, which includes a posteroanterior view of the chest; anteroposterior and lateral views of the cervical spine, thoracic spine, lumbar spine, humeri, femora, and skull; as well as an anteroposterior view of the pelvis. Approximately 80 percent of patients with myeloma will have radiologic evidence of bone involvement on skeletal survey. Although widely employed, this modality has limitations. Roentgenographically detectable osteolytic lesions require at least 50 to 70 percent loss of bone mass<sup>383</sup> and hence represent advanced bone destruction. Conventional x-rays have limited sensitivity and consequently may miss between 10 and 20 percent of

**TABLE 18-4.** Assessment of Myeloma

|                                                                                                                                                     |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|
| Complete blood count and differential count; examination of blood film                                                                              |
| Chemistry screen, including calcium, creatinine, lactate dehydrogenase, BNP, proBNP                                                                 |
| $\beta_2$ -Microglobulin, C-reactive protein                                                                                                        |
| Serum protein electrophoresis, immunofixation, quantification of immunoglobulins, serum-free light chains                                           |
| 24-Hour urine collection for protein electrophoresis; immunofixation; quantification of immunoglobulins, including light chains                     |
| Marrow aspirate and trephine biopsy with metaphase cytogenetics, FISH, immunophenotyping; gene array, and plasma cell labeling index (if available) |
| Bone survey and MRI; PET-CT (if available)                                                                                                          |
| Echocardiogram with assessment of diastolic function and measurement of interventricular septal thickness; ECG (if amyloidosis suspected)           |

BNP, brain natriuretic peptide; CT, computed tomography; ECG, electrocardiogram; FISH, fluorescence *in situ* hybridization; MRI, magnetic resonance imaging; PET, positron emission tomography; proBNP, prohormone B-type natriuretic peptide.

**TABLE 18-5.** Assessment of Myeloma Tumor Mass (Salmon-Durie)

- I. High tumor mass (stage III) ( $>1.2 \times 10^{12}$  myeloma cells/ $m^2$ )\*  
One of the following abnormalities must be present:
- Hemoglobin  $<8.5$  g/dL, hematocrit  $<25\%$
  - Serum calcium  $>12$  mg/dL
  - Very high serum or urine myeloma protein production rates:
    - IgG peak  $>7$  g/dL
    - IgA peak  $>5$  g/dL
    - Urine light chains  $>12$  g/24 h
  - $>3$  lytic bone lesions on bone survey (bone scan not acceptable)
- II. Low tumor mass (stage I) ( $<0.6 \times 10^{12}$  myeloma cells/ $m^2$ )\*  
All of the following must be present:
- Hemoglobin  $>10.5$  g/dL or hematocrit  $>32\%$
  - Serum calcium normal
  - Low serum myeloma protein production rates:
    - IgG peak  $<5$  g/dL
    - IgA peak  $<3$  g/dL
    - Urine light chains  $<4$  g/24 h
  - No bone lesions or osteoporosis
- III. Intermediate tumor mass (stage II) ( $0.6$  to  $1.2 \times 10^{12}$  myeloma cells/ $m^2$ )\*  
All patients who do not qualify for high or low tumor mass categories are considered to have intermediate tumor mass
- No renal failure (creatinine  $\leq 2$  mg/dL)
  - Renal failure (creatinine  $>2$  mg/dL)

\*Estimated number of neoplastic plasma cells.

Reproduced with permission from Durie BG, Salmon SE: A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. *Cancer* 1975 Sep;36(3):842-854.

early lytic lesions.<sup>384</sup> In addition, reproducibility of skeletal survey results is low and dependent on the expertise of the reviewer.<sup>385</sup> Another limitation of plain x-rays is that they cannot be used to assess response to therapy as lytic lesions seldom show evidence of healing.<sup>386</sup> Although skeletal survey remains the gold standard for the initial evaluation of myeloma, there are limitations to this modality that necessitate the use of additional imaging modalities (Fig. 18-14).<sup>387</sup>

**TABLE 18-6.** International Staging System

|            |                                                                |
|------------|----------------------------------------------------------------|
| Stage I:   | $\beta_2M <3.5$<br>ALB $\geq 3.5$                              |
| Stage II:  | $\beta_2M <3.5$<br>ALB $<3.5$<br>or<br>$\beta_2M 3.5$ to $5.5$ |
| Stage III: | $\beta_2M >5.5$                                                |

ALB, serum albumin (in g/dL);  $\beta_2M$ , serum  $\beta_2$ -microglobulin (in mg/L).<sup>231</sup>  
Data from Greipp PR, San Miguel J, Durie BG, et al: International staging system for multiple myeloma. *J Clin Oncol* 2005 May 20;23(15):3412-3420.

MRI is widely used in both newly diagnosed and relapsed myeloma and in the event of suspected cord compression. Whole-body MRI can give complementary information to skeletal survey and is recommended in patients with normal plain radiography, particularly when symptoms are present. Numerous studies demonstrated the superior sensitivity of MRI when compared to both skeletal survey<sup>388,389</sup> and whole-body multidetector computed tomography (MDCT).<sup>390</sup> MRI also has the ability to discriminate myeloma from normal marrow. Specifically, MRI allows visualization of the medullary cavity and thus direct assessment of the extent of myeloma cell infiltration of the bone.<sup>391</sup> In the event of suspected spinal cord compression, MRI is the imaging modality of choice for its ability to provide an assessment of the level and extent of cord compression, the size of the tumor mass, and the extent to which it is compressing the epidural space (Fig. 18-15).<sup>392</sup> In the event that MRI is unavailable or contraindicated, urgent CT may be used for the evaluation of a potential cord compression.

PET, particularly in combination with CT, can be used for the detection of active myeloma. Multiple studies demonstrated that PET-CT is able to detect lesions at least 1 cm in diameter using a standard uptake value (SUV) cutoff of 2.5 to indicate the presence of disease.<sup>393</sup> The limitation of this technology is that subcentimeter lesions may not be detected.<sup>394</sup> In a prospective study comparing PET-CT, MRI, and whole-body x-rays in newly diagnosed myeloma patients, PET-CT was superior to plain films in 46 percent of patients, including 19 percent with negative x-rays. However, PET-CT scans of the spine and pelvis failed to show abnormalities in 30 percent of patients in whom MRI had demonstrated an abnormal pattern of marrow involvement. On the other hand, PET-CT identified myelomatous lesions in areas that were out of the field of view of MRI in 35 percent of patients. The combination of these two modalities proved the most powerful, with a detection rate as high as 92 percent.<sup>395</sup> In a multivariate analysis, the same group also showed that persistent PET-CT positivity before and after primary therapy and subsequent high-dose therapy is a predictor of prognosis in patients with symptomatic myeloma (Fig. 18-16).<sup>396</sup> CT, PET-CT, and MRI can be used for the diagnosis and assessment of soft-tissue masses.

## DIFFERENTIAL DIAGNOSIS

If the initial laboratory evaluation indicates the presence of a monoclonal immunoglobulin in serum and/or urine, the finding requires further studies to distinguish among (1) MG; (2) solitary plasmacytoma of bone or soft tissue; (3) indolent myeloma; (4) immunoglobulin deposition diseases, such as primary amyloidosis or LCDD; and (5) symptomatic or progressive myeloma.<sup>397</sup> Table 18-5 lists the important studies that can be done to distinguish among these possibilities. Tables 18-1 through 18-4 indicate the findings of symptomatic myeloma. The IMWG has developed new standardized diagnostic criteria that have been widely adopted.<sup>398</sup>

## THERAPY

### MANAGEMENT OF NEWLY DIAGNOSED MYELOMA

Myeloma therapy is in a period of dynamic change. The years since 1996 have seen dramatic advances in the treatment of myeloma, beginning with the 1996 publication of a randomized trial investigating the use of high-dose melphalan and autologous stem cell transplant,<sup>399</sup> followed by the introduction of the IMiDs thalidomide,<sup>400</sup> lenalidomide,<sup>401</sup> and pomalidomide<sup>402</sup> and the proteasome inhibitors bortezomib<sup>403</sup>

**TABLE 18-7.** Novel Agent Induction for Newly Diagnosed Transplant-Eligible Patients

| Study                            | Regimen | No. of Patients | Cr/nCR (%) | ORR (%) | Outcome                              |
|----------------------------------|---------|-----------------|------------|---------|--------------------------------------|
| Rajkumar et al. <sup>414</sup>   | RD      | 223             | 18         | 79      | OS 96% on Rd vs. 87% on RD at 1 year |
|                                  | Rd      | 222             | 14         | 68      |                                      |
| Harousseau et al. <sup>638</sup> | VAD     | 121             | 6.4        | 62.8    | PFS 36 mo Bd vs. 30 mo VAD at 32 mo  |
|                                  | Bd      | 121             | 14.8       | 78.5    |                                      |
| Reeder et al. <sup>639</sup>     | CyBorD  | 33              | 39         | 88      | N/A                                  |
| Richardson et al. <sup>418</sup> | RVD     | 66              | 39         | 100     | OS 97% at 18 mo                      |
| Jakubowiak et al. <sup>421</sup> | CRD     | 53              | 62         | 98      | PFS 92% at 24 mo                     |

Bd, bortezomib, low-dose dexamethasone; CRD, carfilzomib, lenalidomide, dexamethasone; CyBorD, cyclophosphamide, bortezomib, dexamethasone; N/A, not available; OS, overall survival; PFS, progression-free survival; RD, lenalidomide, high-dose dexamethasone; Rd, lenalidomide, low-dose dexamethasone; RVD, lenalidomide, Velcade (bortezomib), dexamethasone; VAD, vincristine, Adriamycin (doxorubicin), dexamethasone.

and carfilzomib.<sup>404,405</sup> With these new treatments, the 5-year relative survival rate has increased in the Surveillance, Epidemiology, and End Results (SEER) database from 28.8 percent from the period of 1990 to 1992 to 34.7 percent in years 2002 to 2004 to 40.3 percent in years 2003 to 2007.<sup>406,407</sup> Previously, most of the survival benefit observed was in younger patients, but one analysis showed that patients older than the age of 70 years were deriving benefit as well.<sup>406,407</sup> Table 18-7 lists novel agents and combinations currently used for induction in transplant-eligible patients with newly diagnosed myeloma.

### High-Dose Therapy

Every newly diagnosed myeloma patient should be assessed for fitness to undergo auto-HSCT. Although some centers use an age cutoff (usually age 65 years or younger), it is reasonable to take performance status, organ function, and comorbidities into account, rather than age, when deciding whether a patient is eligible. The rationale for the administration of high doses of alkylating agents (melphalan) followed by transplantation of syngeneic, allogeneic, and autologous marrow or peripheral blood progenitor cells (PBPCs) is based on the fact that myeloma is uniformly fatal, and myeloma cells have demonstrated a dose-response curve to chemotherapy, with a high proportion of patients achieving CRs when higher doses of therapy are given.

High-dose chemoradiotherapy followed by transplantation of either autologous marrow or PBPCs has achieved high (40 percent) CR rates, but the median duration of these responses has been only 2 to 3 years. The Intergroupe Francophone du Myélome (IFM) 90, a national French study, first demonstrated the efficacy of auto-HSCT over conventional chemotherapy in 200 myeloma patients.<sup>399</sup> Several randomized trials and case-controlled studies have been performed, and the results have been variable. For example, the Medical Research Council (MRC) randomized study confirmed a 12-month survival benefit for the transplanted arm.<sup>408</sup> In contrast, the U.S. Intergroup randomized trial was unable to confirm the benefits of transplantation.<sup>409</sup> Despite the use of aggressive approaches like transplantation, few, if any, patients are cured. To improve upon the results of high-dose chemotherapy, the French group has compared single versus double autografts, and their data suggest that two sequential transplants may benefit a subset of patients with myeloma who did not achieve a complete remission after the first transplant.<sup>410</sup> This question is also being addressed by a phase III, multicenter trial of single autologous transplant with or without consolidation therapy versus tandem autologous transplant with lenalidomide maintenance. Preliminary results from this trial show comparable PFS and OS between the three arms. The addition of lenalidomide (Revlimid), bortezomib (Velcade), and dexamethasone (RVD) consolidation or a second auto-HSCT was not superior to a single auto-HSCT followed by lenalidomide maintenance.<sup>410a</sup>

### Novel Agents

Beginning in the 1980s, the combination of vincristine, doxorubicin (Adriamycin), and dexamethasone (VAD) was used as the standard induction chemotherapy, but it has been replaced by the advent of novel drugs.<sup>271</sup> Highly active regimens using IMiDs and proteasome inhibitors have replaced VAD. Two studies combined thalidomide with dexamethasone as initial therapy for myeloma and achieved rapid responses in two-thirds of patients, allowing for successful harvesting of blood stem cells for transplantation.<sup>411,412</sup> Thalidomide/dexamethasone has been compared with VAD and with dexamethasone as initial therapy for patients prior to collection of autologous stem cells and transplantation. In a case-control analysis, thalidomide/dexamethasone achieved higher overall response rates (ORRs),<sup>413</sup> whereas a randomized phase III trial showed statistically significantly higher response rates for thalidomide/dexamethasone than dexamethasone-treated patient cohorts.<sup>271</sup> This study provided the rationale for FDA approval of this regimen for initial treatment of myeloma. Moreover, early studies showed 91 percent responses, including 6 percent complete and 32 percent near-complete/very good partial responses (VGPRs) to lenalidomide combined with dexamethasone (Rd).



**Figure 18-14.** Plain x-rays of osteolytic lesions. Osteolytic lesions captured on plain x-rays or skeletal survey. **A.** Right humerus. **B.** Right femur. **C.** Right radius.



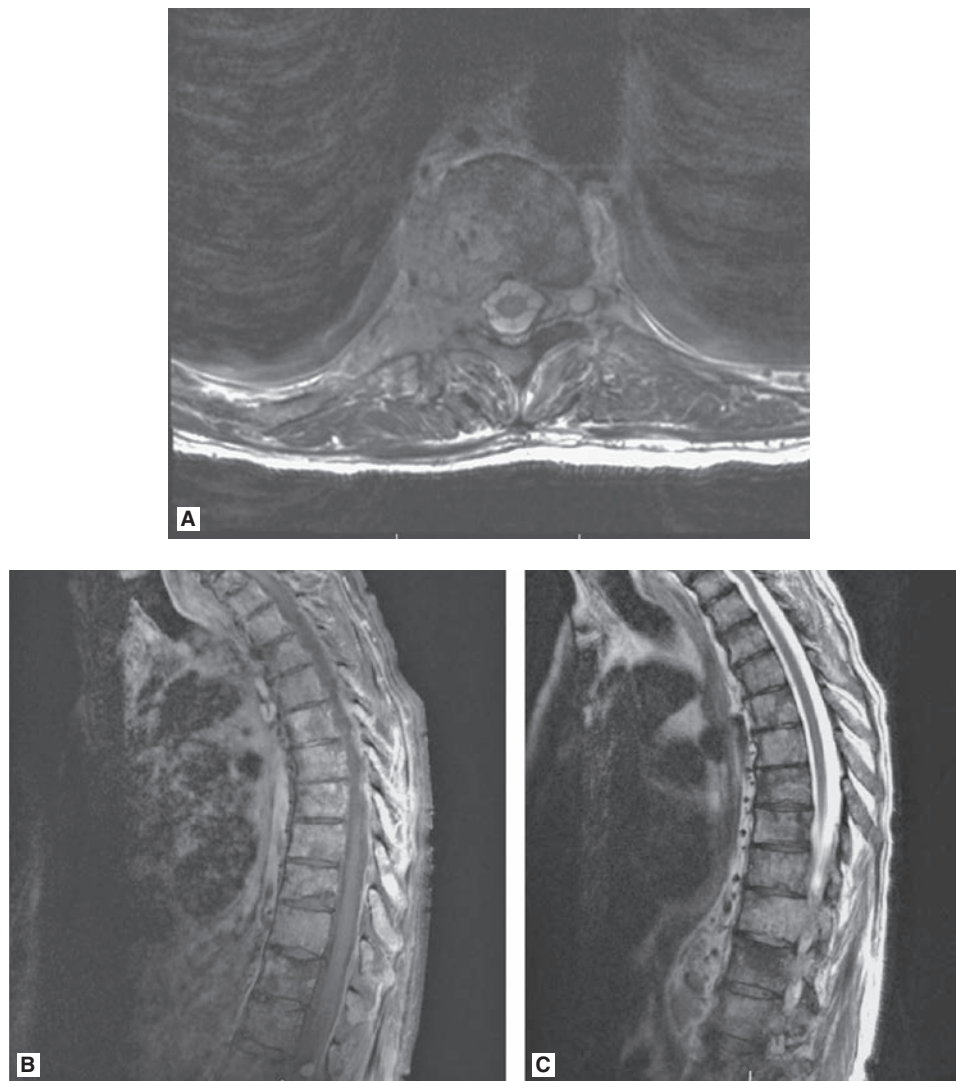


**Figure 18-15** **A.** T2-weighted axial imaging of thoracic spine. Myelomatous lesion involves T11 vertebral body and extends into left posterior elements. **B.** T1-weighted sequencing. Sagittal view of thoracic spine demonstrating diffuse marrow replacement. **C.** T2-weighted sequencing. Sagittal view of thoracic spine. At T10, there is extraosseous extension of soft tissue within the paravertebral space on the right as well as the ventral epidural space and right lateral epidural space. Soft tissue extends laterally to partly efface the right T10–11 neural foramen.

Based on these promising results, a phase III trial in the United States investigated the role of Rd in newly diagnosed myeloma. The study design allowed all patients to stay on-study for the first four cycles only for response assessment, after which patients could go off-study to proceed with SCT. Published safety data from this trial found that combining lenalidomide with the low-dose dexamethasone regimen was preferable to the combination with high-dose dexamethasone, with a reduction in grade 3 or higher nonhematologic adverse events at (48 vs. 65 percent), including thromboembolism (12 vs. 26 percent), and infections (9 vs. 16 percent) in the two treatment arms of the trial.<sup>414</sup> The low-dose dexamethasone-containing regimen did lead to an increased occurrence of grade 3 or greater neutropenia (20 vs. 12 percent). Importantly, the combination with low-dose dexamethasone had a survival benefit over combination with high-dose dexamethasone, with a 1-year OS of 96 and 87 percent, respectively.<sup>414,415</sup> Prophylaxis against clotting with aspirin, Coumadin, or subcutaneous heparin is needed when patients are treated with lenalidomide therapy.<sup>269,270</sup>

One study examined single-agent bortezomib<sup>416</sup> and a second tested bortezomib combined with dexamethasone as initial therapy<sup>417</sup>;

in both studies, high frequency and extent of response were noted. In the phase I/II trials, the safety and efficacy of the RVD combination were demonstrated.<sup>418</sup> The benefits of combination therapy with RVD as first-line therapy were documented in two phase II trials—the IFM 2008 trial and the EVOLUTION trial.<sup>419,420</sup> The ORR after induction in the IFM trial was 97 percent (13 percent sCR, 16 percent CR, and 54 percent VGPR or better). The EVOLUTION trial was designed to compare RVD with cyclophosphamide, bortezomib, and dexamethasone (CyBORd) in a randomized, multicenter setting. The ORR for the RVD arm after primary treatment followed by maintenance with bortezomib for four 6-week cycles was 85 percent (24 percent CR, 51 percent VGPR or better). This finding was confirmed when a subsequent study found a superior PFS, OS, and ORR with the triplet combination of RVD compared to the doublet combination of Rd.<sup>420a</sup>

Carfilzomib is a second-generation proteasome inhibitor that binds to the proteasome in a highly selective and irreversible fashion. Although the drug was initially approved only for relapsed or refractory myeloma, carfilzomib is now being studied in the upfront setting.<sup>404</sup> In a dose-escalation study, the combination of carfilzomib, lenalidomide,

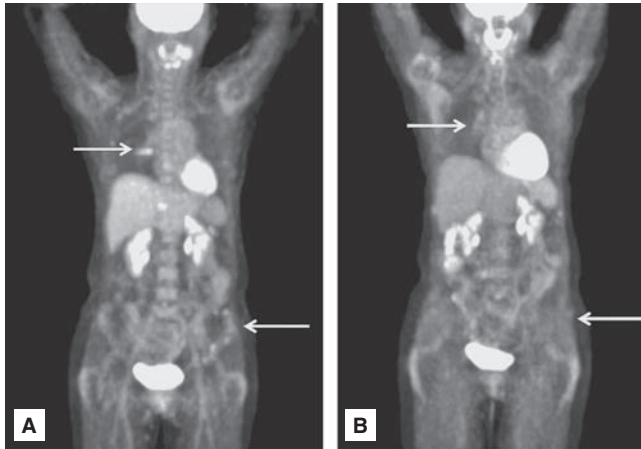


**Figure 18-16.** Positron emission tomographic–computed tomographic (PET-CT) imaging before (A) and after (B) initial therapy for newly diagnosed myeloma with interval resolution of fluorodeoxyglucose uptake.

and dexamethasone (CRD) has been evaluated at carfilzomib doses of 20, 27, and 36 mg/m<sup>2</sup> given on days 1, 2, 8, 9, 15, and 16 for eight cycles followed by days 1, 2, 15, and 16 with subsequent cycles. Lenalidomide was given at a dose of 25 mg on days 1 to 21 and dexamethasone at 40 mg weekly for cycles one to four, then 20 mg weekly for cycles five to eight, with cycles of 28 days.<sup>421</sup> After eight cycles, patients received the regimen every other week for eight cycles. After 24 cycles, maintenance with lenalidomide was recommended off-study. After a median of 12 cycles, 62 percent achieved at least a near CR and 42 percent an sCR. The 24-month PFS was estimated to be 92 percent. The toxicity profile was acceptable and notable for limited peripheral neuropathy. A phase III study is comparing bortezomib, lenalidomide, and dexamethasone to carfilzomib, lenalidomide, and dexamethasone for newly diagnosed disease to determine which is the superior regimen for newly diagnosed patients.

Doublet, and especially triplet, regimens of novel drugs in combination with dexamethasone can induce complete remission rates comparable to transplantation regimens.<sup>413,422</sup> Examples of modern regimens include doublet combinations of Rd and bortezomib and dexamethasone, as well as the triplet combination of RVD, CyBorD, and CRD.

Combinations that include alkylating agents should be avoided as damage to normal hematopoietic stem cells can be incurred, which may render it impossible to collect stem cells for auto-HSCT.<sup>423</sup> Lenalidomide may also hamper the collection of stem cells, although stem cell mobilization with growth factors and chemotherapy may overcome the myelosuppressive effects of lenalidomide.<sup>424–427</sup> The number of cycles of treatment, especially with lenalidomide-containing regimens, is limited to roughly four cycles before stem cell collection, as additional cycles may compromise stem cell harvesting.<sup>424,428</sup>

Combination therapy with novel drugs achieves complete remission rates comparable to those obtained with auto-HSCT. This has led to the design of ongoing studies that compare novel agents followed by auto-HSCT with novel agents followed by delayed auto-HSCT following disease relapse. Novel agents seem to be able to overcome some of the cytogenetic adverse prognostic factors, such as del 13, t(4;14), and del 17p. However, it is premature to abandon auto-HSCT as the followup of clinical trials with new agents is too short to determine whether increased complete remission rates will translate into durable remissions and EFS and OS. Complete remission rates as a surrogate marker for eventual outcome may prove to be inadequate. The IFM 2009 was a phase III, multicenter, randomized trial of RVD versus high-dose treatment with auto-HSCT for myeloma patients up to age 65 years;

it was designed to address the question of the role of autologous transplantation in the context of novel drugs.<sup>422a</sup> Results from this study demonstrated that the median PFS was significantly longer for the group that underwent autotransplant versus the group that received RVD alone (50 vs. 36 months; adjusted hazard ratio [HR] for disease progression or death, 0.65;  $p < 0.001$ ). Furthermore, the percentage of patients achieving a CR was higher in the transplanted group (59 vs. 48 percent,  $p = 0.03$ ). The absence of minimal residual disease (MRD) was also higher in the transplanted group (79 vs. 65 percent,  $p < 0.001$ ). Importantly, 4-year OS did not differ significantly between the two groups (81 vs. 82 percent).<sup>422a</sup> A study in the United States (NCT01208662) will lend further power to the IFM results to assess OS comparing the two treatment approaches.

## THERAPY FOR THE TRANSPLANTATION-INELIGIBLE PATIENT

The traditional age limit for auto-HSCT has been 65 years, although older patients should be considered for transplantation provided good organ function is present. Physiologic rather than chronologic age is more suitable for determining transplantation eligibility.

Oral administration of melphalan and prednisone (MP) has been the standard of care for more than five decades in elderly myeloma patients. This form of therapy produces objective response in 50 to 60 percent of patients. The shortcomings of MP have stimulated investigators to use many combinations of chemotherapeutic agents. Several different combinations have been tested, and two large overviews of more than 10,000 patients have demonstrated that MP had equivalent efficacy and survival to combination chemotherapy.<sup>429,430</sup> Consequently, MP remains a very reasonable treatment strategy for elderly myeloma patients. A number of new approaches promise to be improvements over MP, however. Palumbo and colleagues have incorporated the use of thalidomide in combination with MP in newly diagnosed patients with myeloma who are older than age 65 years.<sup>431</sup> The addition of thalidomide resulted in a 76 percent complete or partial response (PR) rate compared to 47 percent in the MP arm. This translated into a doubling of the 2-year EFS to 54 percent from 27 percent. Based on these data, melphalan, prednisolone, and thalidomide (MPT) emerged as the standard of care for transplantation-ineligible patients. However, all studies showed an increase in adverse events in the MPT arm, including infections, neuropathy, and thromboembolism, suggesting that thromboprophylaxis and antimicrobial prophylaxis is required.<sup>432</sup> Melphalan, prednisone, and lenalidomide (MPR) is another effective regimen in this population. The GIMEMA–Italian Multiple Myeloma Network evaluated 54 patients with this combination. The maximum tolerated dose (MTD) was 0.18 mg/kg melphalan, 2 mg/kg prednisone, and 10 mg lenalidomide. In this study, 81 percent of patients achieved at least a PR, 47.6 percent a VGPR, and 23.8 percent a CR. One-year OS was 100 percent.<sup>433</sup> A subsequent study evaluated the efficacy and safety of induction therapy with MPR followed by lenalidomide maintenance therapy (MPR-R), as compared with MPR or MP without maintenance therapy, in patients with newly diagnosed myeloma who were ineligible for transplantation. At a median followup of 30 months, the median PFS was 31 months for MPR-R versus 14 months for MPR and 13 months for MP. This benefit was observed in patients 65 to 75 years of age, but not in patients older than 75 years. Response rates were superior for the lenalidomide-containing regimen: 77 percent for MPR-R and 68 percent for MPR versus 5 percent with MP.<sup>434</sup>

Randomized trials of other novel agents, like bortezomib with MP, have proven benefits as well. For example, the VISTA trial compared the regimen of bortezomib, melphalan, and prednisone (VMP) to MP in patients who were not candidates for autologous SCT.<sup>435</sup> OS was significantly improved in the VMP group versus MP group, with 3-year OS of 68.5 percent versus 54 percent, respectively.<sup>436</sup>

The FIRST trial, a randomized, phase III trial, compared continuous lenalidomide with low-dose dexamethasone (Rd) against lenalidomide with low-dose dexamethasone for 18 cycles (Rd18) and MPT for 12 cycles.<sup>437</sup> Median PFS for continuous Rd was 25.5 months versus 20.7 for Rd18 and 21.2 for MPT. The OS at 4 years was 59.4 percent for Rd versus 55.7 percent for Rd18 and 51.4 percent for MPT. In the continuous Rd arm, the ORR was 75.1 percent (15.1 percent CR, 28.4 percent VGPR) versus 73.4 in Rd18 (CR 14.2 percent, VGPR 28.5 percent) and 32.3 percent MPT (CR 9.3 percent, VGPR 18.8 percent). The safety profile with continuous Rd was manageable as hematologic and nonhematologic adverse events were as expected for Rd and MPT. Notably, the incidence of hematologic second primary malignancies was lower with continuous Rd than MPT. In newly diagnosed transplantation-ineligible patients, the FIRST trial established continuous Rd as the new standard of care. The combination of bortezomib, lenalidomide, and dexamethasone (RVD lite) at reduced doses and on an attenuated schedule has been evaluated in this population as well. The ORR was 91.4 percent in the 47 patients evaluable for response after four cycles of therapy. Seventy percent of evaluable patients achieved a VGPR or better. Median PFS was 35.1 months (95% CI, 30.9 to  $\infty$ ), and median OS was not reached at a median followup of 30 months.<sup>437a</sup> Table 18–8 provides a summary of clinical trial results for a novel agent induction regimen for newly diagnosed transplantation-ineligible patients.

## MAINTENANCE THERAPY

Maintenance regimens have been proposed to extend the duration of complete remission following autologous SCT. The increased tolerability and efficacy of newer antineoplastic agents have increased the attractiveness and the applicability of this approach; previous attempts at maintenance therapy with older conventional chemotherapy agents such as melphalan or interferon were not beneficial.<sup>438</sup>

A meta-analysis of six randomized, controlled trials with 2786 patients, comparing thalidomide maintenance with other regimens after

induction chemotherapy, demonstrated that patients receiving thalidomide maintenance had marginally better OS (HR 0.83,  $p = 0.07$ ). The difference was most prominent in groups who received both thalidomide and glucocorticoids (HR 0.70,  $p = 0.02$ ). Thalidomide improved PFS (HR 0.65,  $p < 0.01$ ) but was associated with a higher thrombotic risk (risk difference 0.024,  $p < 0.05$ ) and increased peripheral neuropathy (risk difference 0.072,  $p < 0.01$ ).<sup>439</sup>

Three randomized trials explored the use of lenalidomide as maintenance therapy, with two of the trials following autologous SCT<sup>440,441</sup> and one trial after 9 months of melphalan-based therapy in patients ineligible for high-dose treatment.<sup>434</sup> In all three trials, there was a near doubling in PFS with lenalidomide maintenance, for example, from 27 to 46 months in the Cancer and Leukemia Group B (CALGB) 100104 study.<sup>441</sup> Furthermore, the CALGB study showed an OS benefit with lenalidomide: 15 percent of the lenalidomide group had died compared to 23 percent in the placebo group ( $p < 0.03$ ), and at 3 years, the OS was 88 percent in the lenalidomide group compared to 80 percent in the placebo group. In transplant-ineligible patients, the FIRST trial demonstrated somewhat better PFS for those patients receiving continuous lenalidomide maintenance versus a defined course of 18 months of lenalidomide maintenance or MPT (25.5 vs. 20.7 vs. 21.2 months, respectively).<sup>441a</sup>

A significant concern with maintenance therapy with lenalidomide is the risk of secondary malignancy. The secondary cancers observed included both hematologic malignancies, such as acute myelogenous leukemia, and solid tumors. The risk of a secondary hematologic malignancy appears to be greatest when lenalidomide is given in combination with oral melphalan (HR 4.86,  $p < 0.0001$ ).<sup>442</sup> This increased risk of secondary malignancies and the risk-to-benefit ratio of maintenance therapy should be considered and discussed with the patient when initiating maintenance therapy.

Bortezomib has also been studied as maintenance therapy. In the HOVON-65/GMMG-HD4 study, bortezomib was given every 2 weeks and was associated with increasing the near CR and CR rate from 31 to 49 percent.<sup>443</sup> Table 18–9 summarizes maintenance trials.

**TABLE 18–8. Novel Agent Induction for Newly Diagnosed Transplantation-Ineligible Patients**

| Study                    | Regimen | No. of Patients | Median Followup (months) | Median OS (months) | Median PFS (months) |
|--------------------------|---------|-----------------|--------------------------|--------------------|---------------------|
| IFM 99–06 <sup>538</sup> | MP      | 196             | 51.5                     | 33.2               | 17.8                |
|                          | MPT     | 125             |                          | 51.6               | 27.5                |
|                          | MEL100  | 126             |                          | 38.3               | 19.4                |
| IFM 01/01 <sup>640</sup> | MPT     | 113             | 47.5                     | 44                 | 24.1                |
|                          | MP      | 116             |                          | 29.1               | 18.5                |
| MM-015 <sup>434</sup>    | MPR-R   | 152             | 30                       | 45.2               | 31                  |
|                          | MPR     | 153             |                          | NR                 | 14                  |
|                          | MP      | 154             |                          | NR                 | 13                  |
| VISTA <sup>641</sup>     | VMP     | 344             | 60                       | 56.4               | N/A                 |
|                          | MP      | 338             |                          | 43.1               | N/A                 |
| FIRST <sup>437</sup>     | Rd      | 536             | 37                       | 59.4               | 25.5                |
|                          | Rd18    | 541             |                          | 55.7               | 20.7                |
|                          | MPT     | 547             |                          | 51.4               | 21.2                |

MEL100, melphalan 100 mg/m<sup>2</sup>; MP, melphalan and prednisone; MPR, melphalan, prednisone, and lenalidomide; MPR-R, melphalan, prednisone, and lenalidomide induction followed by lenalidomide maintenance; MPT, melphalan, prednisone, and thalidomide; NR, not reached; OS, overall survival; PFS, progression-free survival; Rd, lenalidomide and low-dose dexamethasone continuously; Rd18, lenalidomide and low-dose dexamethasone for 18 cycles; VMP, bortezomib, melphalan, and prednisone.



**TABLE 18–9.** Maintenance Therapies

| Study                            | Regimen                                                                     | No. of Patients | Outcome              |
|----------------------------------|-----------------------------------------------------------------------------|-----------------|----------------------|
| IFM 2005–02 <sup>440</sup>       | Lenalidomide vs. placebo as maintenance following first or second ASCT      | 614             | PFS 41 vs. 23 months |
| CALGB 100104 <sup>441</sup>      | Lenalidomide vs. placebo as maintenance therapy after ASCT                  | 460             | TTP 46 vs. 27 months |
| HOVON-65/GMMG-HD4 <sup>443</sup> | VAD vs. PAD followed by ASCT, then thalidomide or bortezomib as maintenance | 827             | PFS 28 vs. 35 months |

ASCT, autologous stem cell transplantation; CALGB, Cancer and Leukemia Group B; CR, complete response; HOVON-65/GMMG-HD4, Hemato-Oncologie Voor Volwassenen Nederland 65/German Multicenter Myeloma Group High-Dose Melphalan 4; IFM, Intergroupe Francophone du Myélome; PAD, bortezomib, doxorubicin, and dexamethasone; PFS, progression-free survival; TTP, time to progression; VAD, vincristine, doxorubicin, and dexamethasone.

## CONSOLIDATION THERAPY

The use of a short course of consolidation therapy after auto-HSCT increases the CR rate and relapse-free survival. Posttransplantation consolidation using the combination of bortezomib, thalidomide, and dexamethasone made it possible to convert 22 percent of VGPR into full, lasting molecular responses (polymerase chain reaction negative [PCR–]).<sup>444</sup> Enhanced rates of CR, ranging between 10 and 30 percent, have been reported with post–autologous SCT use of bortezomib and lenalidomide as single agents.<sup>445,446</sup> A comparison of melphalan 200 mg/m<sup>2</sup>, auto-HSCT, and four cycles of RVD consolidation versus tandem melphalan 200 mg/m<sup>2</sup> auto-HSCT (TAM) or versus a single auto-HSCT found comparable PFS and OS among the three arms. The addition of RVD consolidation or a second auto-HSCT was not superior to a single auto-HSCT followed by lenalidomide maintenance.<sup>410a</sup>

## CONTINUOUS THERAPY

Data in the upfront setting, both in transplantation-eligible and transplantation-ineligible patients, suggest that continuous therapy may result in improved disease control.<sup>447,448</sup> Several clinical trials have demonstrated superiority of maintenance strategies using thalidomide, lenalidomide, and bortezomib in transplantation-eligible patients.

Thus far, the results from lenalidomide trials are perhaps the most convincing. The IFM 2005–02 and CALGB 100104 studies have both demonstrated a doubling of PFS,<sup>440,441</sup> although only the CALGB trial has suggested an OS advantage. Lenalidomide certainly fits the requirements of a maintenance drug for continued use in myeloma, as it is administered orally and is generally well tolerated. The FIRST trial, comparing continuous Rd with Rd for 18 cycles and MPT, demonstrated that continuous treatment with lenalidomide is superior to finite therapy. PFS for the Rd arm was 25.5 months versus 20.7 months for Rd18 and 21.2 months for MPT.<sup>437</sup> However, the risk of second primary malignancies, although low, does need to be discussed and balanced in the decision-making process when considering maintenance therapy with this agent.

As far as studies of patients with relapsed/refractory myeloma are concerned, treatment generally continues until disease progression.<sup>449</sup> However, the development of toxicities is the biggest challenge of continued therapy in this setting. Ultimately, duration of therapy must be balanced with the adverse events encountered by the patient.

As yet, the optimal duration of therapy for myeloma has not been defined. Data suggest that patients should receive continuous antimyeloma therapy as long as they are benefitting from it and tolerating therapy without excessive toxicity.

## APPROACH TO RELAPSED OR REFRACTORY PATIENTS

A number of options are available for the therapy of relapsing patients. If the relapse occurs more than 6 months after the discontinuation of

the initial therapy, patients may be treated with the same primary therapy. Table 18–10 summarizes trials of novel treatment regimens for relapsed or refractory disease.

### Proteasome Inhibitors

Two phase II studies, SUMMIT and CREST, demonstrated activity of bortezomib in relapsed or refractory myeloma patients.<sup>403,450</sup> An update of the 202 patients enrolled in the SUMMIT study showed median times to progression and duration of response of 7 and 13 months, respectively.<sup>451</sup> A similar analysis of the CREST study demonstrated 5-year survivals of 32 and 45 percent in patients treated with 1.0 mg/m<sup>2</sup> and 1.3 mg/m<sup>2</sup> of bortezomib, respectively.<sup>452</sup> The randomized phase III APEX study comparing bortezomib with dexamethasone alone found a median OS of 30 months in the bortezomib group versus 24 months in the dexamethasone group.<sup>453,454</sup> The addition of dexamethasone to bortezomib monotherapy improved response in 18 to 34 percent of patients.<sup>455</sup>

A next-generation proteasome inhibitor, carfilzomib, was granted accelerated FDA approval as a single agent for the treatment of patients who have received at least two prior lines of therapy; approval was based on the results of the phase II trial of single-agent carfilzomib twice weekly that showed an ORR of 23.7 percent in patients who had had a median of five prior lines of therapy. Median duration of response was 7.8 months, and median OS was 15.6 months. The drug was well tolerated. The most common side effects were fatigue, anemia, nausea, and thrombocytopenia, with 12.4 percent reporting treatment-related peripheral neuropathy and cardiac side-effects.<sup>404</sup>

Oral proteasome inhibitors ixazomib and oprozomib are in clinical trials now and will likely gain approval. Ixazomib has already demonstrated safety and efficacy in phase I trials in the relapsed, refractory population. Of 60 patients who had received a median of six prior regimens including bortezomib (83 percent), there were 41 evaluable patients. Responses included one VGPR, five PR, one minimal response (MR), and 15 with stable disease. Only 10 percent of patients had drug-related peripheral neuropathy, and none was grade 3 or higher.<sup>456</sup> In a phase I/II trial, weekly ixazomib was evaluated in combination with standard dose lenalidomide and dexamethasone in patients with newly diagnosed myeloma. Preliminary results from 58 response-evaluable patients demonstrated a 93 percent ORR, with 67 percent of subjects achieving a very good response rate or better, including a CR rate of 24 percent.<sup>457</sup> Based on these results, ixazomib is being evaluated in combination with lenalidomide and dexamethasone in two large, international phase III trials—TOURMALINE MM1 for relapsed, refractory myeloma patients and TOURMALINE MM2 for newly diagnosed patients. Oprozomib, another oral proteasome inhibitor, is also under investigation for the treatment of myeloma. These drugs could significantly impact the treatment of myeloma, allowing for completely oral treatment regimens and, consequently, completely outpatient care for patients. This could have a measurable quality-of-life benefit for patients, particularly in the elderly population, and may provide a

**TABLE 18–10.** Novel Therapies for Relapsed or Refractory Myeloma

| Trial                            | Phase | Agent         | No. of Patients | ORR (%)         | OS (months) | Outcome (months)                |
|----------------------------------|-------|---------------|-----------------|-----------------|-------------|---------------------------------|
| Richardson et al. <sup>454</sup> | III   | Bortezomib    | 669             | 43              | 29.8        | TTP 6.2 vs. 3.5                 |
|                                  |       | Dexamethasone |                 | 18              | 23.7        |                                 |
| Orlowski et al. <sup>480</sup>   | III   | Bort/PLD      | 646             | 44              | 76%*        | TTP 9.3 vs. 6.5                 |
|                                  |       | Bortezomib    |                 | 41              | 65%*        |                                 |
| Weber et al. <sup>462</sup>      | III   | Lenalidomide  | 353             | 61              | 29.6        | TTP 11.1 vs. 4.7                |
|                                  |       | Dexamethasone |                 | 20              | 20.2        |                                 |
| Dimopoulos et al. <sup>401</sup> | III   | Lenalidomide  | 351             | 60              | NR          | TTP 11.3 vs. 4.7                |
|                                  |       | Dexamethasone |                 | 24              | 20.6        |                                 |
| Richardson et al. <sup>642</sup> | II    | RVD           | 64              | 64              | 26          | Median TTP 9.5                  |
| Siegel et al. <sup>404</sup>     | II    | Carfilzomib   | 266             | 24              | 15.6        | Median PFS 3.7                  |
| San Miguel et al. <sup>643</sup> | III   | Pom/LoDex     | 302             | 31              | 12.7        | Median PFS 4.0 vs. 1.9          |
|                                  |       | Pom/HiDex     |                 | 10              | 8.1         |                                 |
| Dimopoulos et al. <sup>466</sup> | III   | Vor/Bort      | 637             | 56              | NR          | Median PFS 7.6 vs. 6.8          |
|                                  |       | Bort          |                 | 41              | 28.1        |                                 |
| Richardson et al. <sup>467</sup> | III   | Pan/Bort/Dex  | 768             | 61              | NR          | Duration of response 12 vs. 8.1 |
|                                  |       | Bort/Dex      |                 | 55              |             |                                 |
| Lokhorst et al. <sup>470</sup>   | I/II  | Daratumumab   | 32              | 42 <sup>†</sup> | NR          | Median PFS NR                   |
| Lonial et al. <sup>473</sup>     | II    | Elo/Len/Dex   | 73              | 92 <sup>‡</sup> | NR          | Median PFS NR <sup>‡</sup>      |
| Lentzsch et al. <sup>477</sup>   | II    | Benda/Len/Dex | 29              | 52              | NR          | Median PFS 6.1                  |

Benda, bendamustine; Bort, bortezomib; Dex, dexamethasone; Elo, elotuzumab; Hi, high dose; Lo, low dose; NR, not reported/reached; Pan, panobinostat; PLD, pegylated liposomal doxorubicin; Pom, pomalidomide; RVD, lenalidomide, bortezomib, and dexamethasone; Vor, vorinostat.

\*At 15 months.

<sup>†</sup>Of those receiving a dose of  $\geq 4$  mg/kg.

<sup>‡</sup>Of those receiving a dose of 10 mg/kg at 20.8 months.

convenient way of incorporating proteasome inhibitor–based maintenance strategies.

### Immunomodulatory Drugs

Three IMiDs—thalidomide, lenalidomide, and pomalidomide—are FDA approved for the treatment of relapsed or refractory myeloma. Long-term followup of the first thalidomide trial showed that, 10 years after initiation of therapy, 17 of the original cohort of 169 patients were alive, and 10 had an uninterrupted remission.<sup>458</sup> The combination of thalidomide and dexamethasone was superior to dexamethasone alone in several studies.<sup>459–461</sup> Patients who have had prior thalidomide exposure should probably be treated with one of the other novel agents. Furthermore, cytogenetic abnormalities predict for a poor long-term response to thalidomide.<sup>268,458</sup>

Lenalidomide is more potent than its predecessor, thalidomide, and is not associated with sedation, peripheral neuropathy, and severe constipation. In two large, randomized, phase III trials, lenalidomide with high-dose dexamethasone produced a superior response and delayed time to progression compared to dexamethasone and placebo.<sup>401,462</sup> The combination of lenalidomide and dexamethasone had activity in both bortezomib-naïve and previously treated patients, in thalidomide-resistant patients, and after prior auto-HSCT. An analysis of the expanded access lenalidomide program, enrolling 1438 patients, showed that the combination of lenalidomide and dexamethasone had an acceptable safety profile, with less than 10 percent of patients experiencing pneumonia or deep vein thrombosis.<sup>463</sup>

Pomalidomide has also demonstrated potent antimyeloma effects. Several studies have evaluated pomalidomide in combination with low-dose dexamethasone in the relapsed population, culminating in the approval of pomalidomide 4 mg orally on days 1 to 21 of 28-day cycles until progression. The phase II, randomized, open-label study compared pomalidomide and low-dose dexamethasone with single-agent pomalidomide in patients with relapsed or refractory myeloma. Median PFS for the combination arm was 4.2 months compared to 2.7 months for monotherapy (HR 0.68,  $p = 0.003$ ). The ORR was 33 percent with combination therapy versus 18 percent for pomalidomide alone. Median OS was 16.5 months versus 13.6 months, respectively. Refractoriness to lenalidomide and bortezomib did not affect outcomes with pomalidomide and dexamethasone.<sup>464</sup> In the phase III European study comparing pomalidomide and low-dose dexamethasone with high-dose dexamethasone alone, PFS at the interim analysis was 3.6 months in the combination arm compared to 1.8 months for dexamethasone alone (HR 0.45;  $p < 0.001$ ). The most common side effects seen were myelosuppression and infections.<sup>465</sup>

### Histone Deacetylase Inhibitors

Histone deacetylase (HDAC) inhibitors are another class of drugs that have demonstrated activity in relapsed or refractory myeloma when used in combination with bortezomib. The phase III, randomized trial, called Vantage 088, showed the combination of vorinostat and bortezomib was active and well tolerated. When this combination was compared with bortezomib alone, the ORR for vorinostat and bortezomib

was 56.2 percent versus 40.6 percent for bortezomib alone ( $p < 0.0001$ ); similarly, PFS was 7.63 months compared to 6.83 months, respectively ( $p = 0.01$ ).<sup>466</sup> Panobinostat has been combined with bortezomib and dexamethasone in a phase II study and compared with bortezomib and dexamethasone alone in the relapsed/refractory population. PFS was 12 months versus 8.1 months ( $p < 0.0001$ ) for patients treated with the triple therapy. ORR was 61 percent versus 55 percent and duration of response was 13.1 months versus 10.9 months. OS data is not yet mature. Common side effects included myelosuppression and diarrhea.<sup>467</sup> ACY-1215, a selective HDAC 6 inhibitor, is another well-tolerated HDAC inhibitor that is being studied in combination with both lenalidomide and bortezomib plus dexamethasone. The ORR in combination with lenalidomide and dexamethasone was 69 percent even though 13 of 16 patients had received prior lenalidomide and three of six were refractory to lenalidomide.<sup>468</sup> In combination with bortezomib and dexamethasone, the ORR was 60 percent.<sup>469</sup>

### Monoclonal Antibodies

**CD38** Several monoclonal antibodies have demonstrated activity in myeloma, including novel drugs targeting CD38, CS1, and BAFF. Daratumumab, a monoclonal anti-CD38 antibody, was granted Fast Track Designation and Breakthrough Therapy Designation by the FDA based on results of a phase I/II trial that demonstrated single-agent activity in relapsed, refractory myeloma. In the 4 mg or more/kg groups ( $n = 12$ ), five PRs and three MRs were observed. Median PFS had not been reached by a data cutoff of 3.8 months.<sup>470</sup> Based on these encouraging results, daratumumab was combined with lenalidomide and dexamethasone and bortezomib and dexamethasone in relapsed or refractory disease. The bortezomib, dexamethasone, with or without daratumumab (NCT02136134) trial, also referred to as the Castor Trial, was presented at ASCO (American Society of Clinical Oncology) 2016 meeting, favoring the use of triplet combination compared to bortezomib and dexamethasone alone.<sup>470a</sup> One-year PFS for the triplet was 60.7 versus 26.9 percent for bortezomib and dexamethasone alone. The ORR was 83 percent, with 19 percent achieving a CR and 59 percent achieving a VGPR or better. The POLLUX study comparing lenalidomide and dexamethasone alone with lenalidomide, dexamethasone, and daratumumab showed 18-month PFS of 78 percent for the triplet versus 52 percent for the doublet. The ORR was 93 percent, with 43 percent achieving a CR and 33 percent a VGPR.<sup>470b</sup> As a result of these two studies, the FDA has granted Breakthrough Therapy Designation to daratumumab in combination with lenalidomide or bortezomib and dexamethasone for the treatment of myeloma patients who have received one prior line of therapy and in combination with RVD in the frontline setting. The combination of RVD plus daratumumab in the newly diagnosed patient population is currently being evaluated in a phase III clinical trial (NCT02874742). Isatuximab, another anti-CD38 antibody, had an ORR of 30.8 percent as a single agent in the dose-escalation study in relapsed or refractory patients at the MTD.<sup>471</sup>

**SLAMF7** Elotuzumab, a humanized monoclonal IgG<sub>1</sub> antibody directed against human CS1 (also known as CD2 subset 1, SLAMF7, CRACC, and CD319), a cell-surface antigen glycoprotein that is highly expressed on myeloma cells and normal plasma cells, has also been granted Breakthrough Therapy Designation by the FDA. In a phase I study, no objective responses were seen, although 26.5 percent had stable disease by European Bone Marrow Transplant Group (EBMT) myeloma response criteria.<sup>472</sup> In combination with lenalidomide and dexamethasone, objective responses were obtained in 82 percent (23 of 28) of treated patients. After a median followup of 16.4 months, the median time to progression was not reached for patients in the 20-mg/kg cohort who were treated until disease progression. An ORR of 92 percent and a median PFS of 33 months were observed after a median followup of 20.8 months at the 10-mg/kg dose, which was the dose chosen for the phase III trial.<sup>473</sup> Phase I results of elotuzumab in

combination with bortezomib and dexamethasone are also favorable. Phase I results demonstrated a PR or better in 48 percent of 27 evaluable patients with relapsed or refractory disease.<sup>474</sup>

**Programmed Cell Death Protein 1 (PD-1)/Programmed Death-Ligand 1 (PD-L1)** PD-1 is a type I transmembrane protein primarily expressed on activated T cells, B cells, myeloid cells, and antigen-presenting cells.<sup>474a</sup> Binding of PD-1 to PD-L1 and PD-L2 has been shown to inhibit T-cell activation.<sup>474b,474c</sup> Nivolumab was the first anti-PD-1 antibody that has been investigated as monotherapy for myeloma. In a phase I study that included patients with relapsed or refractory lymphoid malignancies, 27 patients with myeloma received nivolumab.<sup>474d</sup> One patient was determined to have a CR after treatment of a rib plasmacytoma; otherwise, durable stable disease was seen.

Pembrolizumab is a humanized IgG<sub>4</sub> monoclonal antibody against PD-1. Patients enrolled in the phase I study of pembrolizumab in combination with lenalidomide and dexamethasone,<sup>474e,474f</sup> had an ORR of 75 percent (24% VGPR, 53% PR). In the lenalidomide-refractory patients, ORR was 50 percent (13 percent VGPR, 35 percent PR). Similar results were seen in the phase I study of pembrolizumab in combination with pomalidomide and dexamethasone, with an ORR of 60 percent.<sup>474g</sup> Combination therapy with PD-1 inhibitors appears to be a promising area of investigation in using the immune system in myeloma therapy.

**B-Cell Maturation Antigen (BCMA)** BCMA (CD269) is a member of the TNF receptor superfamily, TNFRSF17.<sup>474h</sup> Expression of BCMA is restricted to the B-cell lineage, where it is predominantly expressed on differentiated plasma cells<sup>474i</sup> and plasmablasts, making it an attractive target for myeloma therapy.

There is an ongoing phase I study of AMG 224 in RRMM (NCT02561962). AMG 224 is an ADC comprising anti-BCMA-MCC-DM1, where anti-BCMA is an antihuman BCMA IgG<sub>1</sub> antibody; MCC is the noncleavable linker 4-[N-maleimidomethyl] cyclohexane-1-carboxylate conjugated to lysine residues in the antibody; and DM1 is a semisynthetic derivative of the ansamycin antibiotic maytansine conjugated to MCC. Similarly, GSK2857916 is a humanized IgG<sub>1</sub> (ADC) that binds specifically to BCMA and is being investigated in the phase I setting (NCT02064387).

**BAFF** Tabalumab is a fully human monoclonal antibody designed to have neutralizing activity against both membrane-bound and soluble BAFF; it has been combined with bortezomib and dexamethasone. In the phase I/II study, the ORR was 45.8 percent.<sup>475</sup> A phase II trial of this combination has been completed, but results have not yet been reported.

**Bispecific T-Cell Engagers (BiTEs)** BiTEs comprise two single-chain variable fragments, one that binds to CD3 molecules on T cells and the other binds to a surface antigen on the target cells.<sup>475a</sup> MM cell-specific BiTEs are in preclinical development in MM and may offer yet another approach to treating the disease.

### Chimeric Antigen Receptor T-Cell Therapy

Early phase studies are under way examining chimeric antigen receptor (CAR) T-cell therapy in myeloma. Antigens of interest are those whose expression is most limited to plasma cells. Thus far, existing trials have targeted CD19 and BCMA. Though CD19 is rarely expressed on myeloma cells, it is postulated that CD19 might be expressed on a myeloma stem cell, which might be a mature B cell. In the pilot study of anti-CD19 CAR T cell (CTL019) in conjunction with salvage auto-HSCT in advanced myeloma,<sup>475b</sup> 10 subjects received the study therapy. Median number of prior lines of therapy was six. Median PFS after auto-HSCT plus CTL019 was 185 days (range 42–479). In three of 10 subjects, PFS after auto-HSCT plus CTL019 met or exceeded PFS after first-line auto-HSCT. Of the 10 patients treated, six achieved VGPRs by day 100, two had PRs, and two continued to have progressive disease.



Initially, explorations of BCMA-directed CAR T cells have also shown promising results. In the phase I evaluation, six patients were treated.<sup>476</sup> Median number of prior lines of therapy was nine. One patient achieved a stringent CR, which was ongoing at 12 months; one had a VGPR, two MRs, and one stable disease. Depth of response appeared to correlate with degree of CAR-T cells-B-cell maturation antigen (BCMA) expansion and cytokine release syndrome. The limitation of these therapies thus far has been toxicity, including cytokine release syndrome (fever, hypotension, and dyspnea) and prolonged cytopenias. However, the future is promising and may lead to significant improvement in the treatment of myeloma.

### Other Treatments

Bendamustine, as a single agent or in combination with lenalidomide and dexamethasone, is another option for patients with relapsed or refractory myeloma. The combination of bendamustine, lenalidomide, and dexamethasone was evaluated in a phase I/II trial. The median PFS was 6.1 months, PR rate was 52 percent, and the VGPR rate was 24 percent.<sup>477</sup> The MTD of bendamustine was 75 mg/m<sup>2</sup> as compared with prior studies using 100 mg/m<sup>2</sup>. Toxicity was mainly hematologic, arguing in favor of the lower dose, particularly in light of the fact that this population is heavily pretreated.<sup>478</sup>

Other regimens that have been explored include bortezomib and pegylated doxorubicin with or without thalidomide, bortezomib combined with thalidomide and dexamethasone, bendamustine with prednisone and thalidomide, and lenalidomide with doxorubicin and dexamethasone.<sup>479–483</sup> There are currently many new drugs in development that target novel pathways. For example, filanesib, a kinesin spindle protein inhibitor, has demonstrated activity in combination with lenalidomide and bortezomib.<sup>484,485</sup> Several other classes of novel drugs are currently under investigation, including the bromodomain inhibitors, CDK inhibitors, and inhibitors of the ubiquitin pathway. Data generated from these early studies will provide a better understanding of how these new drugs will be incorporated into the continuum of myeloma care.<sup>486–488</sup>

The choice of therapy for relapsed or refractory patients depends on a number of factors, including time since last therapy; prior exposure to novel agents, alone or in combination; and drug-induced comorbidities, for example, neuropathy, renal malfunction, and loss of patient physiologic reserve. In the past decade, there has been a dramatic increase in the number of therapies available to patients with relapsed or refractory myeloma. This is a very dynamic area within oncology and will continue to evolve as more new therapies enter trials and gain approval.

## ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Allogeneic transplantation was seen as an attractive option to treat myeloma because it has the potential to be curative, provides a donor graft that is not contaminated with myeloma stem cells, and may establish a graft-versus-myeloma (GVM) effect that could eradicate any surviving myeloma cells.<sup>489,490</sup> Furthermore, molecular remissions have been observed, which predict for longer survival.<sup>491,492</sup> However, the early experience with myeloablative allogeneic transplantation has not been encouraging as a result of a high mortality, varying from 30 to 50 percent, despite improvements in patient selection and supportive care.<sup>493–500</sup>

Studies from Seattle<sup>494,501</sup> and the EBMT<sup>502</sup> have reported that some patients remain progression free at long intervals after HSCT. The EBMT has reported that actuarial OS was 32 percent at 4 years and 28 percent at 7 years for the 72 (44 percent) patients who achieved CR after allografting.<sup>496,497,503</sup> However, overall PFS was 34 percent at 6 years, and few patients remain in continuing CR for more than 4 years after allograft. Favorable pre-marrow transplantation prognostic factors for both

response and survival after marrow transplantation were female sex, IgA isotype, low serum  $\beta_2$ M, stage I disease at diagnosis, one line of previous treatment, and being in CR prior to marrow transplantation. Of major concern is the early 40 percent transplant-related mortality (50 percent in males) in the EBMT report,<sup>495</sup> which has subsequently been reduced to 20 to 30 percent as a consequence of better patient selection, early transplantation, and less pretransplantation treatment.<sup>503</sup> The actuarial probabilities of survival and PFS were  $0.50 \pm 0.21$  and  $0.43 \pm 0.17$  at 4.5 years. Adverse prognostic factors included transplantation more than 1 year after diagnosis; serum  $\beta_2$ M higher than 2.5 mg/dL at time of transplantation; female patients transplanted from male donors; having received more than eight cycles of chemotherapy; and Salmon-Durie stage III disease at presentation (Table 18–5). Toxicity was substantial, with 35 (44 percent) patients dying of transplant-related causes within 100 days of marrow transplantation.<sup>494,501</sup>

Reduced-intensity conditioning regimens were introduced to reduce transplantation-related mortality and extend the age limit for allografting.<sup>504–509</sup> Autografting has been performed prior to nonmyeloablative transplantation by several investigators, demonstrating the feasibility of this approach to cytoreducing tumor and then enhance antimyeloma immunity.<sup>510,511</sup> Bruno and colleagues published a prospective trial of initial autologous SCT followed by miniallogeneic transplantation versus double autologous transplantation.<sup>512,513</sup> The median OS was 80 months for allografting versus 54 months for double autografting. The CR rate after allografting was 55 percent versus 26 percent after double autograft. In contrast, a randomized trial in high-risk myeloma showed that the combination of autologous SCT followed by dose-reduced allogeneic transplantation (IFM 99–03) was not superior to tandem autotransplant (IFM 99–04).<sup>514</sup> BMT CTN 0102 also evaluated autologous SCT followed by second autologous versus nonmyeloablative allogeneic SCT. In both standard- and high-risk patients, nonmyeloablative allogeneic HSCT after auto-HSCT was not more effective than tandem auto-HSCT.<sup>515,516</sup> Bjorkstrand and colleagues conducted a prospective study of single or tandem autologous SCT versus reduced-intensity allogeneic SCT based on availability of a human leukocyte antigen (HLA)-identical sibling. Long-term followup of 357 patients who received either autologous SCT (single or double) or autologous-allogeneic with HLA-identical sibling matched donor demonstrated that PFS was superior (35 versus 18 percent;  $p = 0.001$ ) at 60 months for those receiving an autologous-allogeneic tandem transplantation. Nonrelapse mortality was 12 percent after autologous-allogeneic versus 3 percent in the autologous group ( $p < 0.001$ ), and the incidence of limited to extensive graft-versus-host disease (GVHD) was 31 percent and 23 percent, respectively.<sup>517</sup>

The use of allogeneic transplantation as salvage therapy, while feasible, is unlikely to be of significant benefit in a heavily pretreated population. The EBMT reported on the outcomes of 229 patients who received reduced-intensity conditioning allogeneic SCT. Transplant-related mortality at 1 year was 22 percent, and 3-year OS and PFS were 41 percent and 21 percent, respectively, with 25 percent of patients developing extensive chronic GVHD.<sup>518</sup> The best outcomes were seen in patients who received a transplant in remission and early in the course of the disease. Adverse OS was associated with chemoresistant disease and transplantation more than 1 year after diagnosis and in male patients transplanted with female donors.

In patients whose disease does not respond to or relapses after allogeneic SCT, donor lymphocyte infusion (DLI) can be considered. Molecular remissions are more common after allografting than after autografting,<sup>492,519–521</sup> and DLI can treat relapsed myeloma after allografting,<sup>490,522,523</sup> indicating a clinically significant GVM effect. In an effort to reduce toxicity and exploit GVM, CD4+ DLIs have been used at 6 months post-CD6-depleted marrow allografting to enhance GVM

and thereby improve outcome.<sup>524</sup> Although prophylactic DLI induces significant GVM responses after allogeneic marrow transplantation, only 58 percent of patients were able to receive DLI despite T-cell-depleted marrow transplantation.

Allografting should only be undertaken in the context of clinical trials, which aim to reduce chronic GVHD, separate GVM from GVHD, and amplify the GVM effect to improve outcome by ameliorating toxicity and maximizing the antimyeloma effect of immunologic effector cells.<sup>525</sup>

## ADJUNCTIVE THERAPIES

### Bone Disease

Bone involvement is one of the defining characteristics of myeloma, either as lytic lesions or diffuse osteopenia. Bisphosphonates, such as pamidronate and zoledronic acid, inhibit osteoclast activity and can palliate pain and prevent bone-related complications.<sup>219,526,527</sup> Hypercalcemia is associated with increased bone resorption in myeloma, and bisphosphonates also play a key role in the treatment of hypercalcemia. Zoledronic acid, which is more potent than pamidronate, is superior to pamidronate for the treatment of hypercalcemia.<sup>528</sup>

The IMWG and the National Comprehensive Cancer Network (NCCN) panels advocate the use of either pamidronate or zoledronic acid monthly for patients with myeloma and lytic bone disease. Both pamidronate and zoledronic acid are considered equally effective in reducing skeletal complications.<sup>529,530</sup> These guidelines recommend the use of pamidronate at 90 mg delivered intravenously for at least 4 hours or zoledronic acid at 4 mg intravenously for 15 minutes every 3 to 4 weeks for patients with myeloma and lytic bone disease.

In addition to playing an important supportive role, bisphosphonates may have a direct antitumor effect. The MRC Myeloma IX trial compared zoledronic acid to oral clodronic acid and found that zoledronic acid reduced mortality by 16 percent and increased median OS from 44.5 months to 50 months.<sup>531</sup> The benefit of zoledronic acid on skeletal morbidity was also seen in patients without bone lesions at baseline.<sup>532</sup>

A key concern with bisphosphonates, especially zoledronic acid, is the risk of osteonecrosis of the jaw (ONJ).<sup>533</sup> In the MRC Myeloma IX trial, the rate of ONJ was 4 percent.<sup>531</sup> Attention to dental hygiene and minimizing invasive procedures may reduce the risk of ONJ.<sup>534</sup>

Denosumab is a monoclonal antibody to RANKL that also inhibits osteoclasts; it showed promising activity in myeloma in a phase II trial.<sup>188</sup> Although denosumab was superior to zoledronic acid in patients with solid tumors and bone metastases, denosumab was inferior in a subset analysis of myeloma patients in a phase III trial.<sup>187</sup> However, interpretation is limited based on the small numbers in the trial. A larger phase III study (NCT01345019) randomized 1718 patients to either zoledronic acid or denosumab. Denosumab was noninferior to zoledronic acid ( $p = 0.01$ ) in delaying time to first on-study skeletal-related event. Fewer adverse events potentially related to renal toxicity were reported with denosumab compared to zoledronic acid overall (10.0 vs. 17.1 percent,  $p < 0.001$ ) in those with baseline CrCl greater than 60 mL/min (8.8 vs. 14.2 percent) and particularly in those with baseline CrCl of 60 mL/min or less (12.9 vs. 26.4 percent). PFS yielded an HR (95% CI) of 0.82 (0.68, 0.99); descriptive  $p = 0.036$ . OS between denosumab and zoledronic acid was HR = 0.9 (0.70, 1.16);  $p = 0.41$ , with fewer deaths in those treated with denosumab (121 [14.1 percent]) than in those given zoledronic acid (129 [15.0 percent]).

Vertebroplasty (injection of methyl methacrylate or bone cement) and kyphoplasty (use of an inflatable balloon followed by instillation of bone cement) are percutaneous procedures for treating compression fractures and have also been used in the setting of myeloma.<sup>535,536</sup>

### Palliative Radiation Therapy

Radiation also plays a key role for palliation of painful bony lesions in myeloma. An estimated 38 percent of patients are expected to receive radiation over the course of their illness.<sup>537</sup> The primary indication for the use of radiation therapy is palliation of bone pain. Other indications include impending fracture, cord compression, or relief of symptoms associated with a mass (i.e., cranial nerve palsies, cosmesis, or organ or joint dysfunction). Doses of 20 to 35 Gy can be used, but it is essential to consider ability to re-treat when designing treatment fields, particularly of the spine. Doses of 20 Gy, delivered in either five or 10 fractions, typically provide adequate symptom relief.

## EMERGENT COMPLICATIONS OF NEW MYELOMA THERAPY

### Venous Thromboembolism

Patients with myeloma are at an increased risk for deep vein thrombosis and pulmonary embolism, particularly when known risk factors are present (history of VTE, immobilization, dehydration, other factors).<sup>432</sup> Genetic predispositions include high levels of homocysteine and deficiencies of antithrombin, protein C, and protein S, as well as mutations that predispose to thrombosis, such as the factor V Leiden allele and/or the prothrombin gene G20210A allele. Genetic abnormalities should be suspected with repeated episodes of VTE. The incidence of VTE is highest during the first 3 to 4 months following diagnosis and occurs in approximately 3 to 4 percent of patients receiving either dexamethasone alone or MP, but is much higher when newer agents are combined with dexamethasone and melphalan.<sup>271,538,539</sup> A number of procoagulant abnormalities have been described in myeloma, including endothelial damage, paraprotein interference with fibrin structure, elevated von Willebrand multimers, elevated factor VIII, decreased protein S, and acquired activated protein C resistance.<sup>540,541</sup> An SNP analysis discovered 18 polymorphisms associated with thalidomide-induced VTE. These polymorphisms were involved in pathways important for drug transport or metabolism, DNA repair, and cytokine pathways.<sup>542</sup> The precise mechanisms causing VTE in myeloma patients absent a known genetic predisposition remain elusive, but the type of therapy plays an important role.

The incidence of VTE with single-agent thalidomide is approximately 2 to 4 percent in newly diagnosed and in relapsed patients, comparable to that observed with dexamethasone alone or MP, implying that thalidomide alone does not increase the risk of VTE. However, the risk for VTE increases significantly when thalidomide is combined with dexamethasone, melphalan, doxorubicin, or cyclophosphamide or with multiagent chemotherapy.<sup>274,432</sup> The MPT combination causes VTE in 12 to 20 percent of patients who do not receive anticoagulant prophylaxis, whereas the incidence of VTE with thalidomide and dexamethasone in newly diagnosed patients is 14 to 26 percent.<sup>271,411,431,538</sup> Most VTEs occur within the first 60 days of therapy, coinciding with maximum cytorreduction. In the Total Therapy 2 study, 22 percent of patients developed a VTE, 95 percent of which occurred in the first 12 months of therapy.<sup>543</sup> Oral regimens principally promote deep vein thrombosis or pulmonary embolism, whereas infusional regimens can give rise to central line–related thrombosis in approximately 50 percent of patients.<sup>542</sup>

Single-agent lenalidomide does not appear to increase VTE, at least not in the setting of myeloma relapse, but is associated with a marked increase in VTE risk when lenalidomide is combined with dexamethasone.<sup>482</sup> Three risk factors for lenalidomide-associated VTE are higher dexamethasone doses, administration of erythropoietin, and concomitant administration of other agents. When combined with cyclophosphamide, the incidence of lenalidomide-induced VTE was

14 percent.<sup>544</sup> Bortezomib did not seem to increase the risk of VTE, at least not in patients with relapsed or refractory disease.<sup>272</sup>

Prevention of VTE is based on the assessment for known risk factors for VTE: (1) myeloma related (hyperviscosity, newly diagnosed status); (2) therapy related (high-dose dexamethasone [ $\geq 480$  mg/month], doxorubicin, multiagent chemotherapy); (3) individual factors (age, history of VTE, inherited thrombophilia, obesity, immobilization, central venous line, infections, surgery, administration of erythropoietin); and (4) factors related to comorbidities (acute infection, diabetes mellitus, cardiac or renal dysfunction). Therapy-related risk factors weigh highest in the risk equation for VTE. The following thromboprophylaxis is recommended: (1) acetylsalicylic acid (aspirin) in either a standard dose of 325 mg/day or in a low dose of 81 mg/day for patients with one or no risk factors or (2) LMWH once a day or full-dose warfarin for patients if two or more risk factors or therapy-related risks are present. The recommended duration of prophylaxis in general is 6 to 12 months.<sup>432</sup>

Therapy for VTE should begin with standard therapeutic doses of LMWH. Oral anticoagulation may be considered as a followup. If oral anticoagulation is deemed inappropriate in a given patient, LMWH should be continued as long as the patient is receiving antineoplastic therapy. The optimal duration of therapy is unknown, but one study reported a recurrence of VTE of 10 percent in patients who had discontinued anticoagulant therapy,<sup>545</sup> suggesting that long-term prophylaxis may be indicated in some patients. A survival benefit for patients who were anticoagulated for a VTE may suggest an additional effect of anticoagulants on the myelomatous process.<sup>543</sup>

### Peripheral Neuropathy

Bortezomib,<sup>546,547</sup> and thalidomide-induced<sup>548,549</sup> peripheral neuropathy should be distinguished from other causes, such as paraneoplastic neuropathies, antecedent chemotherapy with neurotoxic agents (vincristine or cisplatin), diabetes mellitus, and AL amyloidosis. Patients with AL amyloidosis of the peripheral nerves are especially sensitive to neurotoxic agents. Clinical findings include bilateral tingling and numbness in the toes and/or fingers and/or pain ascending in the extremities when neurotoxic drug therapy is continued. Symptoms are typically in a glove-and-stocking distribution. Neurologic examination should evaluate sensory loss, deep tendon reflexes, and distal weakness, especially in the lower extremities. If significant weakness or asymmetry of signs is present, a neurologic consultation must be obtained along with electromyography and nerve conduction studies.

Bortezomib inhibits NF- $\kappa$ B activation, blocking the transcription of nerve growth factor-mediated neuron survival. Other proposed mechanisms for bortezomib-induced neuropathy include mitochondria and endoplasmic reticulum damage because of activation of the mitochondrial-based apoptotic pathway.<sup>546,550,551</sup> Second-generation, more selective proteasome inhibitors such as carfilzomib have reduced neurotoxicity.<sup>552</sup> Grade 3 or 4 bortezomib-induced neurotoxicity occurs in approximately 20 percent of newly diagnosed patients and in 30 percent of patients with relapsing disease.<sup>417,553,554</sup> A 50 percent dose reduction should be made for bortezomib-induced grade 2 neuropathy, while grade 3 or 4 neuropathy requires drug discontinuation. Improvement or resolution of symptoms has been reported in 3 months, whereas in other patients maximum improvement may take 2 years.<sup>403,553,555,556</sup> Subcutaneous dosing of bortezomib appears to be similarly effective as intravenous administration and has a significantly improved safety profile. When compared head to head, 38 percent of patients receiving bortezomib subcutaneously reported any grade of neuropathy compared to 53 percent when given intravenously ( $p = 0.044$ ). By grade, 24 percent versus 41 percent ( $p = 0.012$ ) had grade 2 or worse, and 6 percent versus 16 percent ( $p = 0.026$ ) had grade 3 or worse when comparing subcutaneous with intravenous administration, respectively.<sup>557</sup> Subcutaneous administration is now the preferred route of administration based on these findings.

Daily thalidomide dose, dose intensity, cumulative dose 400 mg or greater, and duration of therapy are all implicated in the pathogenesis of thalidomide-induced neuropathy, which occurs in up to 75 percent of patients.<sup>548,549,558–563</sup> Dose reduction or cessation of therapy with the option of switching to lenalidomide will usually improve neuropathy, although resolution or improvement of neuropathy can take considerable time as thalidomide appears to induce an axonal-length-dependent neuropathy.<sup>564</sup> Symptomatic treatment for thalidomide- and bortezomib-induced neuropathy usually comprises gabapentin, pregabalin, or tricyclic antidepressants.

### Osteonecrosis of the Jaw

ONJ is a severe “bone” disease associated with bisphosphonate therapy that affects the jaws and typically presents as infection with necrotic bone in the mandible or maxilla. ONJ is characterized by the presence of exposed bone in the maxillofacial region that does not heal within 8 weeks. Although asymptomatic at times, ONJ usually presents as pain and/or numbness in the affected area, soft-tissue swelling, drainage, and tooth mobility. The exact cause of ONJ is not known and is likely to be multifactorial. The risk of developing ONJ increases with duration of bisphosphonate exposure and is 5 to 15 percent at 4 years.<sup>565–567</sup> A further predisposing factor for ONJ is invasive dental procedures such as extractions.<sup>568,569</sup> Approximately 50 percent of affected patients had dental work prior to developing ONJ.<sup>569</sup> A genome-wide SNP analysis has shown that a polymorphism in the cytochrome P450-2C polypeptide is associated with an increased risk of developing ONJ on bisphosphonate therapy, although the mechanism underlying this genetic predisposition to ONJ has as yet not been elucidated.<sup>570,571</sup> To prevent ONJ, patients should be referred for dental evaluation prior to commencing intravenous bisphosphonates and should be advised to maintain excellent oral hygiene and avoid dental procedures while receiving these agents.<sup>534</sup> Antibiotic prophylaxis before dental procedures may reduce the incidence of ONJ in patients receiving bisphosphonate therapy.<sup>572</sup> Management is usually conservative (discontinuation of bisphosphonates, limited debridement, antibiotic therapy, and topical mouth rinses).<sup>573</sup> Surgical resection of necrotic bone should be reserved for refractory cases. In a series of 97 patients, healing of ONJ was observed in 75 percent of patients. Patients who develop spontaneous ONJ have a significantly higher risk of nonhealing ONJ or recurrence.<sup>568</sup>

## COURSE AND PROGNOSIS

### MONITORING DISEASE MARKERS FOR DOCUMENTATION OF RESPONSE AND RELAPSE

The previously widely used criteria for assessing response developed by the EBMT Registry, also referred to as the EBMT criteria, have been supplanted by the International Uniform Response Criteria proposed by the IMWG (Table 18–11).<sup>8,574</sup> The IMWG response criteria incorporate the serum-free light-chain assay to allow for assessment of patients previously thought to have hyposecretory or nonsecretory disease. Stricter definitions of complete remission resulted in the inclusion of a category of stringent complete remission in which monoclonal plasma cells are not detectable in the marrow by immunohistochemistry or immunofluorescence and the free light-chain ratio is normal. The previously used near-complete remission (only positivity by serum monoclonal immunoglobulin immunofixation) is now included in the new category of “very good partial response,” and the previously used minor response category is eliminated.

Survival end points include PFS, EFS, and disease-free survival. PFS is the time from start of therapy to myeloma progression or death and includes all patients. It is being used as a surrogate for OS. In the



**TABLE 18–11.** Uniform Response Criteria from the International Myeloma Working Group

| Response Subcategory* | Response Criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|-----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CR                    | Negative immunofixation of the serum and urine and disappearance of any soft-tissue plasmacytomas and <5% plasma cells in marrow. <sup>†</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| sCR                   | CR as defined above plus<br>Normal FLC ratio and<br>Absence of clonal cells in marrow <sup>†</sup> by immunohistochemistry or immunofluorescence. <sup>‡</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| VGPR                  | Serum and urine M protein detectable by immunofixation but not on electrophoresis or 90 or greater reduction in serum M protein plus urine M protein <100 mg per 24 h.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| PR                    | >50% reduction of serum M protein and reduction in 24-h urinary M protein by >90% or to <200 mg per 24 h.<br><br>If the serum and urine M-protein are unmeasurable, >50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria.<br><br>If serum and urine M protein are unmeasurable, and serum-free light assay is also unmeasurable, >50% reduction in plasma cells is required in place of M protein, provided baseline marrow plasma cell percentage was >30%.<br><br>In addition to the above listed criteria, if present at baseline, a >50% reduction in the size of soft-tissue plasmacytomas is also required. |
| SD                    | Not meeting criteria for CR, VGPR, PR, or progressive disease.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

CR, complete response; FLC, free light chain; M protein, monoclonal protein; PR, partial response; sCR, stringent complete response; VGPR, very good partial response.

\*All response categories require two consecutive assessments made at any time before the institution of any new therapy; CR, PR, and stable disease categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed. Radiographic studies are not required to satisfy these response requirements.

<sup>†</sup>Confirmation with repeat marrow biopsy not needed.

<sup>‡</sup>Presence/absence of clonal cells is based upon the  $\kappa:\lambda$  ratio of >4:1 or <1:2. An abnormal  $\kappa:\lambda$  ratio by immunohistochemistry and/or immunofluorescence requires a minimum of 100 plasma cells for analysis.

Note: SD is not recommended for use as an indicator of response; stability of disease is best described by providing the time-to-progression estimates.

case of EFS, precise definition of what constitutes an event is required (e.g., significant drug toxicity, death, etc.). Stable disease is no longer used as a measure of treatment efficacy, but rather time to progression, which is measured from the start of therapy and, importantly, includes all patients entered into clinical studies. Duration of a response is calculated from the onset of response and only counts the subgroup of responding patients. Long-term followup is recommended to fully evaluate the impact of novel treatment. Other limitations of the new criteria are that response is determined only by monoclonal immunoglobulin and marrow evaluation. Dynamic changes in skeletal events readily

identified by modern imaging techniques such as MRI and PET-CT are excluded from response assessments.

Disease features can change over the course of the disease. Not infrequently, clonal evolution occurs with successive relapses, resulting in the loss of previously secreted intact immunoglobulin and a switch to secretion of light chains only (“Bence Jones escape”) or entire loss of immunoglobulin-secretory capacity, often associated with extramedullary spread, best signified by increased LDH levels and lesions found on PET-CT examination. Occasionally, unexplained anemia or pancytopenia accompanies the disappearance of myeloma protein markers, necessitating prompt marrow examination to detect fulminant relapse.

Many induction regimens currently affect tumor cytorreduction rapidly, so that monoclonal immunoglobulin reduction of 50 percent or more is apparent within a few months of therapy. Thus, at least monthly myeloma protein evaluations should be performed during induction. After two to four induction cycles and prior to high-dose melphalan-based auto-HSCT, the disease is restaged to include marrow examination with cytogenetics and MRI and/or PET-CT of indicator lesions in order to assess whether intramedullary or EMD has been reduced. Disease monitoring should be performed at least every month for the first year and at a minimum every other month thereafter. Marrow biopsy, including cytogenetic examinations, should be performed at the time of progression or change in therapy. Imaging should be performed annually or in the presence of new symptoms.

### Minimal Residual Disease

Sequencing-based platforms, quantitative polymerase chain reaction, and multiparametric flow cytometry are now being employed to detect MRD in patients attaining at least a VGPR after primary therapy, which may be of significant prognostic value. Patients who achieve an MRD-negative state have a much better prognosis. A meta-analysis of 21 studies included 14 that evaluated the impact of MRD on PFS and 12 that evaluated MRD and OS in newly diagnosed patients. Those patients who achieved an MRD-negative status had a PFS that was nearly twice as long as those who were MRD positive (54 vs. 26 months,  $p < 0.001$ ). OS was also longer for MRD-negative patients (98 vs. 82 months,  $p < 0.001$ ). The study also examined the relationship between MRD status and PFS and OS in those achieving a CR and found that the benefit persisted among those attaining a CR.<sup>574a</sup>

In 2016, the IMWG issued consensus criteria incorporating MRD assessments into the response criteria.<sup>574b</sup> The goal of this addition was to define a response state that reflects a higher degree of tumor eradication, which better correlates with prognosis. The goal of capturing MRD data is not only for prognostication but also for the development of response-adaptive treatment strategies. MRD testing also has high potential as a surrogate end point for survival and may be a pathway for clinical studies directed at obtaining accelerated approval for new myeloma therapies.<sup>575</sup> The best method for performing MRD analysis and the best use of the test continue to evolve, but MRD is a key variable in assessing myeloma therapy, and MRD increasingly is incorporated into study design.

## PROGNOSIS

The prognosis of myeloma is determined by three factors: (1) the host, (2) tumor biology and disease burden, and (3) the type of therapy applied. Host parameters, such as comorbidities, advanced age, frailty, and poor performance status, negatively impact overall outcome and increase treatment-related morbidity and mortality. With the advent of new drugs, used in combination or incorporated into high-dose melphalan-based auto-HSCT approaches, some poor prognostic factors can be overcome (see “Management of Newly Diagnosed Myeloma”).

Advances in cytogenetics, gene-expression profiling, and imaging (MRI and PET-CT) have greatly increased our understanding of tumor

biology and burden. Furthermore, such variables are more powerful prognostic indicators than standard prognostic variables.

Numerous individual parameters have been examined for their value as prognostic features. Higher labeling indices, serum IL-6 receptor levels, increased *RAS* mutations, more aggressive disease, and shortened survival have been reported in patients with plasmablastic morphology.<sup>292</sup> Serum  $\beta_2$ M represents the light chain of the MHC complex of the cell membrane. Increased serum  $\beta_2$ M results from secretion by tumors with a high growth fraction and rapid cell turnover rates. In patients with myeloma and normal renal function, rising serum  $\beta_2$ M predicts progression.<sup>301</sup> The LI, a measure of DNA synthesis by myeloma cells, predicts survival. It is usually low (<1 percent) at diagnosis, higher at relapse, and lower in MG and indolent myeloma.<sup>576</sup>

Serum IL-6 levels appear to correlate with both stage of disease and survival.<sup>577,578</sup> IL-6 stimulates hepatocytes to produce acute-phase proteins, such as CRP; CRP, therefore, may reflect the IL-6 level and proliferative status of marrow plasma cells. Indeed, CRP levels are significantly lower in patients with MG than in those with MM, and survival can be correlated with serum CRP level.<sup>579</sup> High serum levels of soluble IL-6 receptor (sIL-6R), hepatocyte growth factor,<sup>580</sup> and syndecan-1,<sup>581</sup> as well as low serum levels of hyaluronate,<sup>582</sup> are independent prognostic factors predicting poor outcome.

The percentage of circulating plasma cells in the blood and their labeling indices are independent prognostic factors for survival in myeloma after both conventional and high-dose therapy.<sup>294,583</sup> Circulating endothelial cells also correlate with disease course and response to thalidomide.<sup>584</sup> Finally, circulating proteasome levels are an independent prognostic factor for survival.<sup>585</sup>

Many of these factors are interrelated and therefore of limited independent value. Using multivariate analysis, several groups have found that the best combination of variables to predict outcome was serum  $\beta_2$ M (reflecting both the tumor burden and the renal function) and the proliferative activity of plasma cells, evaluated by the LI or number of tumor cells in S phase. Age and performance status also improve the prognostic assessment.<sup>586,587</sup>

Cytogenetic findings associated with poor outcome include hypodiploidy and deletion 17p13, the locus of the tumor-suppressor gene *TP53*.<sup>308,309,372,588</sup> Gains of chromosome 1q arm and loss of 1p occur in tandem duplications and jumping translocations of chromosome 1 and signify more aggressive and more advanced myeloma.<sup>59,589–591</sup> Combining hypodiploidy and high  $\beta_2$ M has also been used to identify patient populations with a poor outcome.<sup>588,592</sup> In contrast, hyperdiploidy, which accounts for almost half of the patients with abnormal cytogenetics and involves nonrandom gains of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, is associated with chemosensitive disease and better OS. Translocation t(11;14) also confers a better outcome.<sup>101</sup> Chromosome 13 deletions are present in more than 50 percent of patients with myeloma and are associated with poor prognosis; however, these deletions are also associated with MG,<sup>83,89,307</sup> and their role in transformation to myeloma is undefined at present. Chromosome 13 deletion is not prognostic for response to bortezomib, highlighting the importance of prognostic factors for particular therapies.<sup>593</sup>

Interphase FISH does not depend on cycling cells and increases the detection of abnormal cells to 80 to 90 percent.<sup>594</sup> Interphase FISH can also detect cytogenetically silent translocations, for example, t(4;14), t(14;16), and t(14;20), and can be performed on stored material. The data just given suggest that analysis of metaphase cytogenetic abnormalities and FISH can identify patients who do not do well with auto-HSCT or standard therapies. Individual prognostication remains highly variable despite the application of these more sophisticated cytogenetic techniques. The Total Therapy 2 patient data set revealed that standard

prognostic variables and metaphase cytogenetics had limited ability to account for outcome variability with HRs not exceeding 2.0.<sup>64,595,596</sup>

Gene-expression profiling on newly diagnosed patients has allowed for the interpretation of outcome in the context of whole human genomic data. Gene-expression profiling has the ability not only to define disease pathogenesis but also to identify both novel prognostic factors and potential therapeutic targets.<sup>597,598</sup> Using tumor cells from 532 newly diagnosed patients, Shaughnessy and colleagues defined a 70-gene model and a 17-gene subset that have the ability to identify high-risk disease.<sup>599</sup> In a study by the IFM, gene-expression profiles were generated for 250 newly diagnosed patients. The 15 most stable genes were used to construct a survival model. The 15-gene model demonstrated an improved ability to predict survival in newly diagnosed patients with myeloma treated with high-dose therapy. Specifically, this work demonstrated that high-risk patients have a 6.8-fold HR (95 percent confidence interval, 3.92 to 11.73;  $p < 0.001$ ) of death compared with low-risk patients. IFM 15-gene and University of Arkansas for Medical Sciences (UAMS) 17-gene models, when applied to their respective data sets, are powerful prognostic tools that can enhance the ISS for the identification of high-risk disease. The HOVON group developed a 92-gene signature that predicts for differential outcome.<sup>600</sup> These gene signatures are, however, not yet generalizable, as they have not been studied prospectively. Moreover, an analysis of signatures defined in Arkansas, IFM, Millennium, and HOVON studies showed that these signatures are not broadly useful in myeloma.<sup>129</sup>

These gene-expression profiling studies also identified mechanisms of sensitivity versus resistance to conventional and novel myeloma therapies.<sup>601,602</sup> For example, gene-expression profiling of patient tumor samples showed genes upregulated in patients responding to bortezomib compared to patients who did not respond. Hsp27 upregulation correlated with intrinsic or acquired bortezomib resistance (Chap. 20). Preclinical studies showed that p38<sup>MAPK</sup> inhibition down-regulated Hsp27 expression and restored Velcade sensitivity in resistant myeloma cell lines and patient samples,<sup>603</sup> providing the basis for a trial combining these two agents.

Gene-expression profiling data should have an important impact on the interpretation of future clinical trials. Validated prognostic models from gene microarray data are now used for improved prognostication and will ultimately be used to assist in selecting therapy for individual patients.

Myeloma is a very complex disease at the genomic level. Parallel sequencing of paired and normal samples from 203 myeloma patients were performed, and mutations were most frequently observed in *KRAS*, *NRAS*, *BRAF*, *FAM46C*, *TP53*, and *DIS3*.<sup>112</sup> The tumors demonstrated significant heterogeneity. Mutations were often present in subclonal populations, and multiple mutations within the same pathway were observed within the same patient. Results of whole-exome sequencing, copy number profiling, and cytogenetics of myeloma samples, including serial samples in 15 patients, demonstrated complex clonal evolution over time.<sup>108</sup> Moreover, clonal and subclonal heterogeneity within the same patient without the predominant clone did not necessarily translate into mRNA. These and other studies demonstrated the complexity of the myeloma genome, underscoring a need for rapid identification of possible druggable oncogenic mutations to customize therapy for myeloma patients. Genomic analysis technology that can facilitate this understanding may prove valuable in the further development of effective targeted therapies. Future strategies of treatment may include the combination of targeted therapies with existing proteasome inhibitors and IMiDs. In addition, identification of mutations may provide important prognostic information. For example, *SP140* mutations were associated with increased risk of relapse, suggesting that this gene may have prognostic features in myeloma.

## SPECIAL DISEASE MANIFESTATIONS

### IGM MYELOMA

A rare diagnostic dilemma concerns the existence of an IgM myeloma entity that is distinct from Waldenström macroglobulinemia (histopathologic diagnosis, immunocytoma).<sup>604,605</sup> Upon examination, plasma cells, rather than the lymphoplasmacytic infiltrate, are seen to dominate the marrow of myeloma, whereas mastocytosis is a hallmark of immunocytoma. DNA aneuploidy and the presence of lytic bone lesions support a diagnosis of myeloma. Myeloma, also of the IgM isotype, is resistant to purine analogues, which are effective in Waldenström macroglobulinemia.<sup>606,607</sup>

### SOLITARY PLASMACYTOMA

Plasmacytomas are collections of monoclonal plasma cells originating either in bone (solitary osseous plasmacytoma [SOP]) or in soft tissue (extramedullary plasmacytoma [EMP]). They comprise less than 10 percent of plasma cell dyscrasias. Indications of systemic disease, such as marrow plasmacytosis, anemia, renal insufficiency, or multiple lytic or soft-tissue lesions, must be excluded before the diagnosis of either SOP or EMP can be made. MRI can be useful to show additional marrow abnormalities consistent with myeloma.<sup>608</sup> The median age of diagnosis of either SOP or EMP is approximately 50 years, nearly 10 years younger than that for myeloma.<sup>609–611</sup> Although patients with SOP and EMP can progress to myeloma, persons with SOP progress in the majority of cases, in contrast to EMP, where only 50 percent eventually develop myeloma. The median survival of 86.4 months and 100.8 months for patients with SOP and EMP, respectively, is similar; however, PFS is markedly different, 16 percent for SOP patients versus 71 percent for EMP patients. The persistence of stable monoclonal immunoglobulin in the serum and/or urine after primary treatment of plasmacytoma does not necessitate additional therapy, as it does not influence survival or disease-free survival.<sup>609</sup> In contrast, rising monoclonal immunoglobulin levels in a patient with a history of either SOP or EMP should trigger a workup for either recurrent plasmacytoma or myeloma. It has been suggested, as is true for myeloma, that serum  $\beta_2$ M has prognostic value in patients with SOP. For example, 17 of 19 patients with elevated serum  $\beta_2$ M had transformation to myeloma and shorter survival (31 months) as compared with those with normal serum  $\beta_2$ M levels.<sup>612</sup>

Local therapy, primarily radiotherapy, with surgery as needed for structural anatomic support is the standard treatment for SOP and EMP.<sup>609–611</sup> Patients with soft-tissue solitary plasmacytomas can often be cured with appropriate local radiation (dose of at least 4.5 Gy). By contrast, this local treatment approach fails in the majority of patients with solitary plasmacytomas of bone.<sup>613</sup> The development of myeloma in such patients probably reflects multifocal systemic disease present at the outset and hitherto not detectable by standard radiographic imaging but readily by applying MRI<sup>614</sup> and PET-CT.<sup>615</sup>

The benefit of chemotherapy, either alone or in combination with radiotherapy and surgery, as primary therapy for SOP or EMP has not been proven. Moreover, the benefit of adjuvant chemotherapy, given to prevent recurrent disease and/or progression to myeloma, is also undefined. Disappearance of protein after involved-field radiotherapy predicts for long-term disease-free survival and possible cure.<sup>613</sup>

### AMYLOID LIGHT-CHAIN AMYLOIDOSIS

When clinical features of congestive heart failure, nephrotic syndrome, malabsorption, coagulopathy, skin rash (oral mucosal rash, “raccoon’s

eyes”), or neuropathy are present, a careful search for primary amyloidosis should be carried out (Chap. 19). LCDD may also have a similar clinical presentation. The primary difference between AL amyloidosis and LCDD is the difference in structure of the deposited protein; in AL amyloidosis, it is fibrillar versus granular in LCDD. LCDD is usually associated with the  $\kappa$  light-chain subtype, whereas AL amyloidosis is associated with the  $\lambda$  light-chain subtype of myeloma.

Primary AL amyloidosis and immunoglobulin deposition diseases are best characterized functionally as MG with clinical manifestations because of normal tissue infiltration by these processes, although they can also accompany overt myeloma. Further workup following suspicion on clinical presentation depends on the organ of concern. Cardiac amyloidosis may be associated with low voltage on an electrocardiogram, arrhythmias, increased interventricular septal thickness greater than 12 mm, diastolic dysfunction or speckling on echocardiogram, and elevation of markers, such as B-type natriuretic peptide, N-terminal pro-B-type natriuretic protein, and cardiac troponin T.<sup>616,617</sup> Involvement of the gastrointestinal tract may present with decreased albumin and prealbumin. Renal involvement may present as nonspecific proteinuria with high total protein on 24-hour collection and low monoclonal immunoglobulin. Carpal tunnel syndrome and peripheral neuropathy may be a manifestation of amyloid, and nerve conduction studies may help in this diagnosis. Orthostatic hypotension also should alert to the possibility of systemic amyloidosis as a result of amyloid deposition in vasa nervorum of the autonomic nervous system or in adrenal glands, resulting in hypoadrenalism. Occasionally, primary amyloidosis presents as tumors either mostly consisting of amyloid or mixed with plasmacytoma. Typically, MRI signals can distinguish plasmacytomas, which show hypointensity on T1-weighted images and hyperintensity on short tau inversion recovery (STIR)-weighted images, whereas amyloidoma-type lesions remain hypointense.

Appropriate biopsy techniques should be applied to clarify the presence and extent of accompanying amyloidosis or, similarly, LCDD in these patients. The diagnosis of AL amyloid (Chap. 19) often can be made by fine-needle aspiration of subcutaneous fat or by biopsy of the rectal mucosa,<sup>618</sup> although biopsy of accessible clinically involved tissue is preferable. AL amyloid also may be detectable on marrow biopsy.<sup>252</sup> Staining the tissue with Congo red may reveal perivascular amyloid with its classical apple-green birefringence when viewed under polarized light.<sup>619</sup> Thioflavin T is also a useful stain, producing intense yellow-green fluorescence in AL amyloidosis. LCDD requires immunofluorescence analysis of unfixed tissue; formalin fixation should be avoided whenever it is suspected.

Even though the tumor load is very low, patients with AL amyloidosis and immunoglobulin deposition disease suffer from the consequences of myeloma secretory products, even at relatively modest amounts, resulting in damage to kidneys, heart, gastrointestinal tract, liver, spleen, and peripheral and autonomic nerves. All current treatment targets the monoclonal plasma cell population, and advances have paralleled the advances in treatment of myeloma. Whereas standard melphalan-prednisone has been only marginally effective, high-dose dexamethasone pulsing plus interferon, effecting more rapid and profound responses in myeloma, has also shown encouraging results in AL amyloidosis.<sup>620</sup> Similarly, positive results have been obtained with dexamethasone plus melphalan.<sup>621</sup> The Boston University group has pioneered the use of high-dose melphalan with auto-HSCT (see Fig. 18–16),<sup>622</sup> which is an effective regimen in carefully selected patients. In a study of 312 patients, high-dose melphalan (100 to 200 mg/m<sup>2</sup>) with auto-HSCT resulted in a median survival of 4.6 years with a treatment-related mortality of 13 percent.<sup>623</sup> There was also significant improvement in organ function. The role of allogeneic transplantation in AL amyloidosis is unclear.



Incorporation of the newer agents, such as the IMiDs and proteasome inhibitors, has shown promising results in the treatment of patients with AL amyloidosis in combination with other agents, such as melphalan, dexamethasone, and cyclophosphamide. In phase II trials of lenalidomide in combination with dexamethasone, 29 percent achieved a CR and 38 percent achieved a PR with an overall response of 67 percent. Lenalidomide was given at a dose reduction of 15 mg/day because of poor tolerability at a standard dose of 25 mg/day.<sup>624</sup> The safety and efficacy of the combination of pomalidomide and dexamethasone was also evaluated in a phase II clinical trial. Of 33 patients, the ORR was 48 percent. The time to response was 1.9 months, and the median OS and PFS rates were 28 and 14 months, respectively. The most common side effects were fatigue and neutropenia.<sup>625</sup>

Bortezomib has also demonstrated efficacy and tolerability in the treatment of AL amyloidosis. In a phase II trial of 40 patients not achieving a CR after autologous SCT, 21 patients were treated with bortezomib and dexamethasone. Of the 12 evaluable patients at 1 year post-autologous SCT, ORR was 92 percent—67 percent achieved a CR and 50 percent had organ responses.<sup>626</sup> When used in combination with cyclophosphamide (CyBorD), high response rates are reported. Venner and colleagues evaluated the combination in 43 treatment-naïve patients. The overall hematologic response rate was 81.4 percent, with CRs in 39.5 percent; 30 percent of patients reported peripheral neuropathy. This is likely attributable to the fact that dosing on this regimen was biweekly, and the route of bortezomib administration was intravenous.<sup>627</sup>

Cardiac amyloid remains the most challenging clinical condition and is currently addressed with repeated cycles of dose-reduced melphalan (70 to 100 mg/m<sup>2</sup>) and stem cell support to avoid cardiac catastrophes possibly linked to arrhythmias caused by fluid overload or cytokines.<sup>628</sup> There is a prevailing misconception that high-dose dexamethasone pulsing, alone or with added thalidomide, even at low doses, is safer and better tolerated than appropriately dosed melphalan with stem cell support. Owing to its hematopoietic stem cell-compromising properties, melphalan, even at 50 to 70 mg/m<sup>2</sup>, is still best administered in the context of autologous stem cell support.

## SMOLDERING MYELOMA

Patients with smoldering myeloma have historically been followed without therapy.<sup>629–631</sup> At this time, there is no role for treating smoldering myeloma patients. Patients should be followed at close intervals of every 3 to 6 months, and clinical trials should be offered where available.

At a median followup of 40 months in a small, randomized, prospective study of 125 patients with high-risk smoldering myeloma, treated with either lenalidomide and dexamethasone or observation,<sup>632</sup> TTP was not reached by the intervention arm compared to 21 months in the observation arm ( $p < 0.001$ ), and the 3-year OS was 94 percent versus 30 percent ( $p = 0.03$ ), respectively. Despite these favorable findings, the high-risk criteria employed by this study are not those commonly used in practice. High risk in this study was defined as having at least 10 percent marrow infiltration with plasma cells, an M protein of 3 g/dL or greater, or a urinary Bence Jones protein level of more than 1 g/24 hours. Based on these criteria, some patients with active myeloma were classified as having high-risk smoldering myeloma, which may have contributed to the differences in the outcomes between the two arms of the trial. The IMWG has advised that those smoldering myeloma patients who have greater than 60 percent marrow plasma cells,<sup>633</sup>  $\kappa:\lambda$  FLC ratios greater than 100,<sup>634</sup> and two lesions on MRI or PET-CT imaging may benefit from therapy.<sup>635–637</sup> Further investigation of treatment in high-risk myeloma is warranted before changing the treatment paradigm in this population.

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## CHAPTER 19

# IMMUNOGLOBULIN LIGHT-CHAIN AMYLOIDOSIS

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### SUMMARY

Amyloidosis should be considered in any patient presenting with nephrotic range proteinuria; infiltrative cardiomyopathy or heart failure with preserved ejection fraction; hepatomegaly without specific imaging findings; or peripheral neuropathy, particularly if a monoclonal protein is present; as well as any patient with atypical multiple myeloma.

When a patient is seen with a relevant syndrome, the patient should have immunofixation of serum and urine proteins and measurement of  $\kappa$  and  $\lambda$  immunoglobulin free light chains. If all of these tests are normal, it is unlikely that the patient has immunoglobulin light-chain (AL) amyloidosis.

If any of the tests mentioned are positive, further investigation for amyloidosis should be undertaken. The diagnostic test of choice is subcutaneous fat aspiration; marrow biopsy is the second best procedure. With these two tests, 83 percent of patients will have a positive result when their aspiration or biopsy material is stained with Congo red under green birefringence.

All patients with biopsy-proven amyloidosis should have the deposits analyzed by laser capture microdissection mass spectroscopy to definitively classify the exact protein subunit composing the amyloid. This technique does not distinguish between systemic and localized amyloidosis, however.

The prognosis in AL amyloidosis is determined by three tests: (1) assessment of the N-terminal probrain natriuretic peptide, (2) assessment of serum troponin, and (3) the difference between the involved and uninvolved immunoglobulin free light chains. These three tests can be combined to stage the patient from stage 1 through stage 4.

Treatment of AL amyloidosis involves either standard systemic chemotherapy or high-dose chemotherapy with autologous stem cell transplantation. Fit patients who are expected to have low morbidity with transplantation should undergo this approach. The majority of patients, however, will not be candidates for transplantation and should be treated with traditional systemic chemotherapy, with the cyclophosphamide, bortezomib, and dexamethasone regimen currently favored by many investigators.

### DEFINITION AND HISTORY

Amyloidosis is a heterogeneous group of diseases characterized by tissue infiltration with misfolded protein precursors as amyloid material. For nearly 100 years, amyloid has been defined by its staining properties. Divry and Florkin first used Congo red to detect amyloid in the

brain of patients with Alzheimer disease.<sup>1</sup> Amyloid deposits are always extracellular and, under the light microscope, appear amorphous and pink when seen after standard hematoxylin-and-eosin staining. All amyloid deposits bind Congo red dye and demonstrate green birefringence under polarized light,<sup>2</sup> and this test remains the standard diagnostic procedure required to confirm a diagnosis of amyloidosis. Under the electron microscope, amyloid consists of rigid, linear, nonbranching fibrils of 9.5-nm diameter.<sup>3</sup> Historically, amyloid was defined based on whether there was a family history (inherited), whether it was the result of a chronic inflammatory condition (secondary), or whether it was idiopathic (primary). Today, amyloidosis is classified based on the protein subunit composition of the deposits. The term *primary amyloid* is archaic and should not be used. Instead, such cases should be called *immunoglobulin light-chain (AL) amyloidosis*, reflecting the fact that they represent a systemic plasma cell dyscrasia. The subunit classification is important because amyloid A (AA) amyloidosis (secondary) not only can be the consequence of sustained inflammation but also can be inherited, as in familial Mediterranean fever. Inherited forms of amyloidosis are generally composed of mutant transthyretin (TTR) but can also be a consequence of mutations in apolipoprotein, fibrinogen, and gelsolin.<sup>4</sup> Senile systemic amyloidosis, primarily a cardiac disorder, is being increasingly recognized and is caused by deposition of wild-type TTR as amyloid material in patients older than age 60 years.<sup>5</sup> Table 19–1 lists the nomenclature of the various forms of amyloidosis. Practicing hematologists and oncologists are not likely to see patients with forms of amyloidosis other than AL amyloidosis. This is the only form that is responsive to chemotherapy and is the focus of this chapter; in addition, the term *amyloidosis*, unless specified otherwise, refers to AL amyloidosis.

### EPIDEMIOLOGY

Amyloidosis is rare, with an incidence of eight per million persons per year and with a median age at diagnosis of 67 years.<sup>6</sup> This makes it one-sixth as common as myeloma and twice as common as Waldenström macroglobulinemia. Conversely, a practicing oncologist should see approximately one light-chain amyloid patient for every six patients with myeloma in the oncologist's practice. If this is not the case, there is a significant likelihood that the disease is going unrecognized.

### ETIOLOGY AND PATHOGENESIS

The fibrils of immunoglobulin light-chain amyloid are composed, in most patients, of fragments of immunoglobulin light chains. A normal immunoglobulin light chain has a molecular weight of approximately 25 kDa, but the immunoglobulin light chains found in amyloid deposits usually range from 8 kDa to 15 kDa. The fragment size is important because it usually reflects the deletion of the constant region of the immunoglobulin light chain, making immunohistochemistry a poor technique to identify the fibril type in paraffin-embedded tissues.<sup>7</sup> A fraction of patients have immunoglobulin heavy-chain amyloid deposits, where fragments of immunoglobulin (Ig) G, A, or M heavy chains comprise the basic subunit. Whereas AL amyloidosis represents approximately 62 percent of all amyloid patients, immunoglobulin heavy-chain amyloidosis represents less than 1 percent of all patients.<sup>8</sup>

There is clearly something “amyloidogenic” about the immunoglobulin light chains in patients with AL amyloidosis. Unlike monoclonal gammopathy and myeloma, where the  $\kappa$ : $\lambda$  ratio is 2:1, in AL amyloidosis, the  $\kappa$ : $\lambda$  ratio is 1:3, suggesting an intrinsic propensity of  $\lambda$  light chains to form amyloid. Moreover, the subgroup  $\lambda_{\text{VL}}$  is exclusively found in patients with AL amyloidosis.<sup>9</sup> When the light chains are extracted from the urine

**Acronyms and Abbreviations:** CRAB, calcemia, renal insufficiency, anemia, or bone disease; CyBorD, cyclophosphamide, bortezomib, and dexamethasone; MRI, magnetic resonance imaging; NT-ProBNP, N-terminal probrain natriuretic peptide; TTR, transthyretin.

**TABLE 19–1. Nomenclature of Amyloidosis**

| Amyloid Type                       | Subunit Protein                                                     | Clinical Organ Involvement            |
|------------------------------------|---------------------------------------------------------------------|---------------------------------------|
| AL ( $\kappa$ or $\lambda$ ) or AH | Immunoglobulin light or heavy chain<br>May be localized or systemic | Heart<br>Kidney<br>Liver<br>Nerve     |
| AA                                 | Secondary serum amyloid A                                           | Kidney<br>Gastrointestinal<br>Thyroid |
| ATTR (age related)                 | Senile systemic transthyretin                                       | Heart<br>Carpal tunnel                |
| ATTR (mutant)                      | Familial transthyretin                                              | Heart<br>Nerve                        |
| A Lect-2                           | Leukocyte chemotactic factor<br>No mutation found                   | Kidney                                |
| A Ins                              | Insulin localized to injection sites                                | Skin                                  |
| A Fib                              | Fibrinogen A-2 mutation                                             | Kidney                                |
| A $\beta_2$ M                      | $\beta_2$ -Microglobulin<br>Chronic dialysis                        | Soft tissue<br>Joints spine           |

of patients with myeloma and injected into mice, amyloid deposits are not seen. However, when the light chains from patients with amyloidosis are extracted from the urine and injected into mice, they will develop amyloidosis.<sup>9</sup> The exact structural characteristics that lead to the misfolding of the  $\alpha$ -helical protein into the amyloid  $\beta$ -pleated sheet configuration are unknown. Patients with amyloidosis are classified into those with myeloma and those without myeloma. The percentage of plasma cells present in the marrow at the time of diagnosis is prognostic and predicts outcome in patients with amyloidosis.<sup>10</sup> As a result, the percentage of plasma cells in the marrow may dictate alternate therapeutic considerations at diagnosis (see “Therapy” section). Overt myeloma with CRAB (hypercalcemia, renal insufficiency, anemia, or bone disease) criteria is uncommon in AL amyloidosis. However, an elevation in the percentage of plasma cells in the marrow of patients without CRAB criteria confers the same adverse prognosis as observed in patients who have overt symptomatic myeloma, and these groups can be considered as a single cohort of those with myeloma-associated amyloidosis. Patients who do not have myeloma at presentation have almost no chance of developing myeloma later in the course of their disease.<sup>11</sup> The plasma cells in the marrow of patients with amyloidosis tend to be nonproliferative and frequently lack the karyotypic abnormalities typically seen in myeloma, such as  $-17p$ ,  $t(4;14)$ , and  $-13$ .<sup>13</sup> A translocation  $11;14$ <sup>14</sup> is commonly seen in amyloidosis and appears to confer an inferior outcome. Circulating plasma cells detectable by flow cytometry are uncommonly seen relative to their frequency in myeloma.<sup>12</sup>

## CLINICAL FEATURES

Unfortunately, the symptoms of amyloidosis are vague and often nonspecific. Physical findings, when present, can be highly specific but are only present in a minority of patients. For a disease this rare, it

is important for clinicians to have an operational approach to avoid inappropriate resource utilization. Only 1 percent of patients with AL amyloidosis are younger than age 40 years. Consequently, it would be unlikely for younger patients to have AL amyloidosis, although this age group regularly can be seen with secondary systemic amyloidosis. The majority of patients with AL amyloidosis are males (66 percent), as in myeloma, the male-to-female ratio is 52:48.<sup>15</sup>

The most common symptoms reported by patients with amyloidosis are fatigue, weight loss, and lower extremity edema. Unfortunately, these symptoms are too nonspecific to be used as a trigger to screen patients, as the yield for these vague symptoms would be very low. The mechanism underlying fatigue in this disorder is usually amyloid heart disease, which can be an extremely subtle diagnosis and is discussed further under the specific amyloid syndromes. Weight loss accompanying amyloidosis can be impressive and may exceed 20 kg. This usually results in an investigation for occult malignancy, but even if blind biopsies are performed, the diagnosis may be overlooked if a specific request for amyloid stains is not made to the pathologist. Lower extremity edema may be attributable to nephrotic range proteinuria, hypoalbuminemia, and transudation of serum into the extracellular space. Edema may also be seen from high filling pressures caused by the restrictive cardiomyopathy, which, again, can easily be overlooked because of the subtle diagnostic testing required. These patients are often treated empirically with diuretics until cardiac dysfunction becomes more evident.

The physical findings of amyloidosis are present in only one patient in six. Periorbital purpura is frequently cited as a diagnostic finding and, when present, is useful but is only seen in 15 percent of patients (Fig. 19–1). Sometimes, the purpura is subtle and can only be seen with the patient's eyes closed. Even when purpura is present, it may lead to an evaluation of a coagulopathy or platelet dysfunction, which would invariably be negative in this setting. Purpura is seen not only on the eyelids but also on the face, neck, and anterior chest above the nipple line. Enlargement of the tongue is seen in approximately one person in eight (Fig. 19–2). Dental indentations on the underside of the tongue are characteristic. Often, the patient will have sudden onset of sleep apnea syndrome as a result of occlusion of the airway by the enlarged tongue when the patient is supine.<sup>16</sup> Even when diagnostic findings are present, their significance is often missed, leading in many cases to tongue biopsy with a preoperative suspicion of tongue cancer. Most patients with enlargement of the tongue will also have palpable submandibular lymphadenopathy, usually caused by displacement of



**Figure 19–1.** Amyloid purpura.





**Figure 19-2.** Macroglossia as a result of amyloid infiltration.

the nodes by the enlarged base of the tongue, although amyloid infiltration of the submandibular gland may also result in a firm palpable mass in the submandibular region. Hepatomegaly is seen in approximately 20 percent of patients and may be due to direct infiltration of the liver, which will cause a firm, markedly enlarged liver.<sup>17</sup> In some patients, however, the liver enlargement is a reflection of high venous filling pressures in the right-sided cardiac chambers and represents chronic passive congestion. Patients rarely have periarticular infiltration of the shoulders, producing the so-called shoulder pad sign, a baseball-shaped enlargement of the anterior soft tissues of the shoulder. A rare patient will develop temporal artery infiltration and develop classic jaw claudication, as well as limb, buttock, and calf claudication.<sup>18</sup> On questioning, many patients will have xerostomia from infiltration of the minor salivary glands; and at some centers, biopsy of the minor salivary glands is a preferred technique for the diagnosis of amyloidosis.<sup>19</sup>

## ● AMYLOID SYNDROMES

Because the symptoms of amyloidosis are highly nonspecific and the physical findings are specific but not very sensitive, an operational approach is required to ascertain which patients need investigation for amyloidosis. We recommend screening for AL amyloidosis when a patient is seen with any of the following clinical syndromes:

- Nephrotic range proteinuria with any serum creatinine level
- Infiltrative cardiomyopathy or heart failure with preserved ejection fraction; a normal ejection fraction does not exclude AL amyloidosis
- Hepatomegaly or alkaline phosphatase elevation without specific imaging abnormalities
- A mixed axonal demyelinating peripheral sensory, motor, or autonomic neuropathy, particularly when associated with a monoclonal gammopathy
- A patient with myeloma with symptoms that are not typical of the disease, particularly profound, unexplained fatigue

**TABLE 19-2.** Amyloid Syndromes Seen at the Mayo Clinic

|                  | % Present |
|------------------|-----------|
| Kidney           | 67        |
| Heart            | 47        |
| Peripheral nerve | 12        |
| Liver            | 12        |
| Autonomic nerve  | 4         |
| Carpal tunnel    | 12        |
| Tongue           | 9         |

Table 19-2 gives the frequency of amyloid syndromes seen in patients at the Mayo Clinic. If an oncologist sees a patient with one of these five syndromes, or an internist is faced with an undiagnosed patient, laboratory evaluation is indicated, as outlined next.

## LABORATORY FEATURES

The best screening tests for initial evaluation of patients with suspected amyloidosis are immunoelectrophoresis and immunofixation of both serum and urine and a serum immunoglobulin free light-chain assay (both  $\kappa$  and  $\lambda$ ). Systemic immunoglobulin AL amyloidosis is a plasma cell dyscrasia, and 99 percent of patients will have a detectable abnormality of one of these three tests, reflecting synthesis by a clonal population of plasma cells in the marrow. If an immunoglobulin protein is detected, further investigation for amyloidosis as described in the next section ("Differential Diagnosis") should proceed. If a systemic plasma cell dyscrasia and an immunoglobulin light chain cannot be confirmed, three possibilities exist: (1) The patient does not have amyloidosis; (2) the patient does not have systemic amyloidosis; or (3) the amyloidosis is not immunoglobulin light chain in type and reflects a different protein subunit.

The serum-free light-chain assay is a critically important test. It not only heightens the suspicion of the presence of immunoglobulin AL amyloidosis, but also is prognostic and vital to staging the patient. The serum immunoglobulin free light chain is part of the response evaluation for this disease. A screening serum protein electrophoresis is insufficient as a screening technique because a visible M spike is seen in less than half of patients because of the high prevalence of primary light-chain proteinemia.

Finding a monoclonal protein in the serum or in the urine of a patient with heavy albuminuria often obviates the need for a renal biopsy. A patient with free light chains in the serum or urine and proteinuria can have only one of three disorders: (1) myeloma cast nephropathy, (2) AL amyloidosis, or (3) Randall-type immunoglobulin deposition disease ( $\kappa$ ).

Screening for a light-chain immunoglobulin is the best noninvasive approach when confronted with a patient with any of the seven syndromes listed in Table 19-2. If amyloid is present but the light chains are normal, strong consideration of referral to a specialty center to further clarify the underlying form of amyloidosis should be considered.

## DIFFERENTIAL DIAGNOSIS

Once a clinician has begun an evaluation of a patient with a compatible clinical syndrome and an immunoglobulin abnormality has been detected, a biopsy is required to confirm the diagnosis before therapy should commence. Although imaging of amyloid deposits with

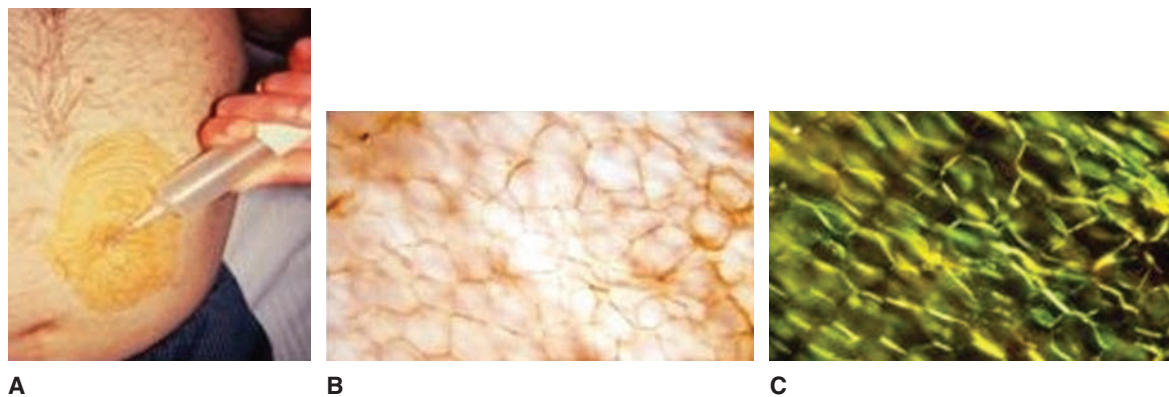


**Figure 19-3.** Technique and results of subcutaneous fat aspiration. **A.** Procedural technique. **B.** Fat stained with Congo red; note the preserved interstices of the fat cells. **C.** Viewed under polarized light to demonstrate green birefringence.

various radionuclides, most notably anti-serum amyloid P component, continues to be explored and used in practice, it is not a substitute for histologic confirmation of the presence of amyloidosis.<sup>20–22</sup> In a patient with renal, cardiac, hepatic, or peripheral nerve amyloidosis, it is straightforward to establish the diagnosis by kidney, heart, liver, or nerve biopsy, but this is unnecessary in the majority of patients. Subcutaneous fat aspiration is a bedside office procedure done with local anesthesia and does not require an incision (Fig. 19–3). An excellent YouTube video has been produced by the Boston Medical Center on the technique (<https://www.youtube.com/watch?v=tctYTmxd9gQ>). Typically, fat aspirations are reviewed at a specialty center unless the pathology department regularly processes fatty tissue. The fat is not paraffin embedded; therefore, it is subject to overfixation, trapping of Congo red dye, and the possibility of inadequate controls.<sup>23</sup> Fat aspiration is positive in three-quarters of patients with amyloidosis.

A second tissue easily stained for amyloid is the marrow. Because AL amyloidosis patients will have a plasma cell dyscrasia, a marrow examination is clinically indicated to quantify the number of plasma cells in the marrow. Congo red staining of marrow blood vessels can detect amyloid deposits in half of patients with AL amyloidosis. Combining the two techniques (marrow and fat pad aspiration) establishes a diagnosis in 85 percent of afflicted patients. Minor salivary gland biopsy, endoscopic gastric biopsy, rectal biopsy, and skin biopsy can also be used to establish the diagnosis. If the clinical suspicion of AL amyloidosis is strong and these biopsies are negative, it is appropriate to biopsy the affected organ.

Once tissue containing Congo red has been identified, it becomes imperative that the protein subunit be determined. Historically, immunohistochemistry has been used to identify the type of amyloid, but immunohistochemistry can be challenging because only those protein subunits for which antisera exist can be detected.<sup>24,25</sup> Second, particularly in AL amyloidosis, the reactive epitope may have been deleted during the process of amyloid fibril formation, and, frequently, the background staining is high, particularly in kidney tissues. Third, because the protein in amyloid misfolds, even if the epitopes are present, they may be hidden deep within the deposits and may be inaccessible to commercial antisera. Not only can immunohistochemistry be unreliable, but also it can be misleading. It is therefore recommended that laser capture of the amyloid deposit be performed routinely followed by mass spectroscopic analysis.<sup>26–28</sup> The subunit protein can be identified by this approach in the majority of cases. Even in patients who have a false-positive Congo red stain, this technique is useful because it will not identify amyloid-related peptides upon analysis. With this technique, one is able to confirm the presence of AL amyloidosis, which represents slightly

less than two-thirds of patients seen with amyloidosis. A full quarter of patients with amyloid deposits are composed of amyloidogenic transthyretin (ATTR) (inherited) or wild-type amyloid (senile systemic). Less than 4 percent are amyloid A (AA) and approximately 4 percent represent ALect2 amyloidosis, which deposits in the kidney and causes proteinuria. Other forms of amyloid, which are often localized, are seen in less than 1 percent of patients. A particularly important form of localized amyloid occurs at the sites of subcutaneous insulin injections in diabetics. Crystalline insulin can form amyloid, which can cause discolored firm deposits that, when biopsied, will be Congo red positive but by mass spectroscopic analysis can be confirmed to be insulin. Other forms of systemic amyloidosis for which systemic chemotherapy is contraindicated include fibrinogen A- $\alpha$  amyloidosis and amyloid caused by apolipoprotein A1 and gelsolin.

One additional advantage, particularly for those patients who have TTR amyloidosis, is that mass spectroscopy not only identifies the protein, in most instances, it can confirm whether the protein is wild type or a mutant. We still recommend that patients who have mutant TTR seen on mass spectroscopy have this confirmed by DNA sequencing of a blood sample, which is a readily available commercial test, to confirm the findings on mass spectroscopy. We have investigated a small number of patients in whom an amyloid syndrome was strongly suspected, a subcutaneous fat aspirate was not definitively positive, yet mass spectroscopic analysis demonstrated peptides in the tissue that are associated with amyloid, including apolipoprotein E and serum amyloid P component. When a pathologist makes a diagnosis of amyloid in tissue sections, the clinicians are required to ask what type of amyloidosis it represents. Although not widely available, mass spectroscopic analysis is the most sensitive and specific technique for identifying the amyloid subunit protein.

## DIAGNOSIS AND CLINICAL CHARACTERIZATION OF ORGAN-SPECIFIC SYNDROMES

### Kidney

Kidney involvement is seen in 45 percent of patients with immunoglobulin AL amyloidosis. It is conspicuously absent in the majority of patients with TTR amyloidosis until late in the disease. Renal involvement is virtually universal in systemic AA amyloidosis as well as ALect2 amyloidosis and A-fib  $\alpha$  amyloidosis. Amyloid is seen in 2.5 percent of all renal biopsies. When limited to nondiabetics older than age 50 years, amyloid deposits will be found in 10 percent of renal biopsies.<sup>29</sup>



These patients present with the typical four features of the nephrotic syndrome: (1) nephrotic range proteinuria, (2) hypoalbuminemia, (3) hyperlipidemia, and (4) edema. Nearly a third of patients with renal amyloidosis have at least a 1-year history of dramatic elevations of cholesterol and triglycerides. These are often managed with statin-type medication and dietary modification without consideration that a dramatic ( $>100$  mg/dL) rise in cholesterol and triglycerides may be caused by heavy proteinuria. In the majority of patients, the glomerular filtration rate (GFR) is preserved until sustained proteinuria has been present for years. Only a small percentage of patients, usually with interstitial but not glomerular amyloid, present with renal insufficiency in the absence of heavy proteinuria.<sup>30</sup>

The management of the lower extremity edema is primarily diuretic therapy. However, excessive diuretic use, particularly in patients with cardiac amyloidosis, can aggravate already reduced intravascular volume. Diuretics can also compromise renal blood flow, increase orthostatic hypotension, and reduce cardiac-filling pressures necessary for adequate cardiac output in patients that have “stiff heart syndrome.” The threat of continuous proteinuria in amyloidosis is damage to the tubular system. One-third of patients with renal amyloidosis will ultimately require dialysis or renal transplantation.<sup>31</sup> The serum creatinine level at the time of presentation is the best predictor of which patients are most likely to require dialysis. Clearly, the best method for prevention of the need for dialysis is effective therapy of the underlying plasma cell dyscrasia. There are no reported differences between outcomes for those patients receiving hemodialysis and those receiving peritoneal dialysis.

In rare instances, patients have profound depression of the serum albumin below 1 g/dL. These patients often will be disabled by anasarca. In situations where intractable edema and anasarca make management next to impossible, renal ablation has been performed to stop the urinary protein leak, normalize the serum oncotic pressure, and resolve the edema. Multiple techniques have been reported, including nephrectomy, ligation of the renal artery, and bilateral ureteral clips. In some patients, the early initiation of dialysis, even with a normal estimated GFR, can result in anuria and restoration of normal serum albumin levels.<sup>32</sup>

The correlation between the amount of amyloid detected and the degree of renal dysfunction is poor. The urinary sediment is nonspecific, shows fat and fatty casts, but generally does not contain red cell casts. The most common cause of death in patients with renal amyloidosis is progressive cardiac dysfunction from infiltrative amyloid cardiomyopathy.

### Heart

Unlike most cardiac disorders, which are caused by a loss of systolic function, the restrictive cardiomyopathy caused by amyloid infiltration results in poor relaxation (so-called stiff heart syndrome) and poor filling during diastole so that the ventricular chamber has a low end-diastolic volume, which results in reduced stroke volume and reduced cardiac output. This constitutes a classic example of heart failure with preserved systolic function. In fact, the majority of patients with amyloidosis have an echocardiographically normal ejection fraction until late in the disease.<sup>5</sup> Cardiac amyloid is found in 40 to 50 percent of patients at diagnosis and is responsible for nearly 90 percent of deaths. It is the most challenging syndrome to diagnose because of the lack of specificity of its symptoms. Fatigue and dyspnea on exertion are often not associated with cardiac disease in the presence of amyloid (1) because of the lack of radiographic changes of cardiomegaly, pleural effusions, and pulmonary vascular redistribution; (2) because echocardiography will demonstrate preserved ejection fraction; and (3) because coronary angiography is invariably normal.<sup>33</sup> This triad often leads to a diagnosis of noncardiac dyspnea. Even when amyloid infiltrates the myocardial wall and causes

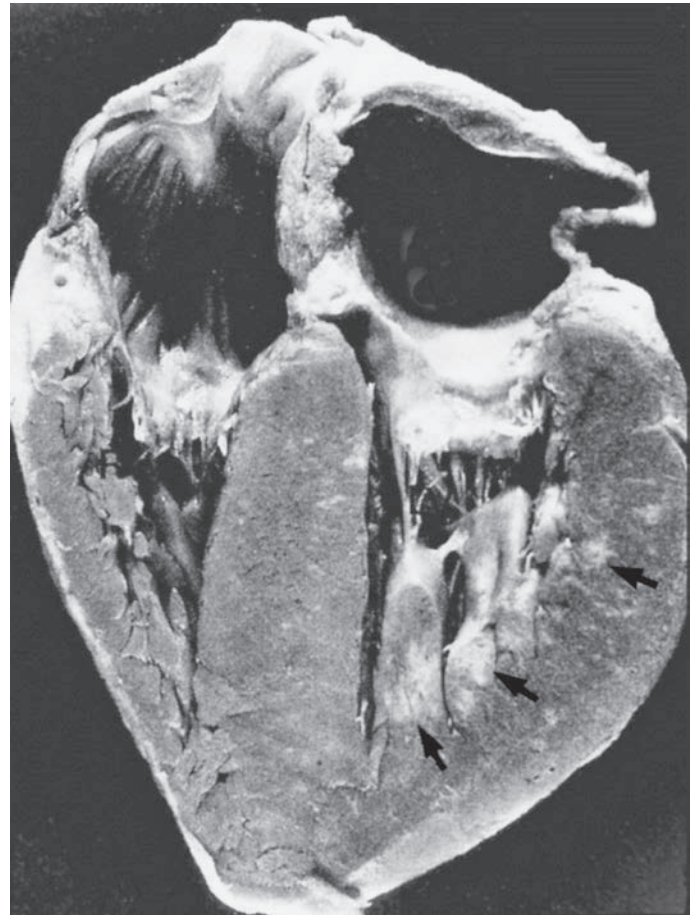


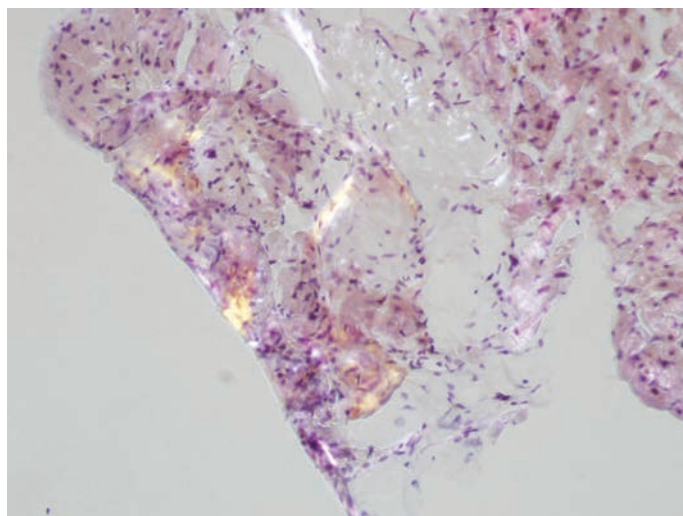
**Figure 19-4.** Amyloid heart disease. Note thickening of heart walls from infiltration. White patches were responsible for the designation “lardaceous change.”

it to be thickened, it is often misattributed to hypertensive heart disease (hypertrophy) rather than infiltration (Fig. 19-4). Electrocardiographic abnormalities, including pseudoinfarction and low voltage, are quite common but are frequently overlooked, whereas a pseudoinfarction pattern is misattributed to ischemic heart disease.

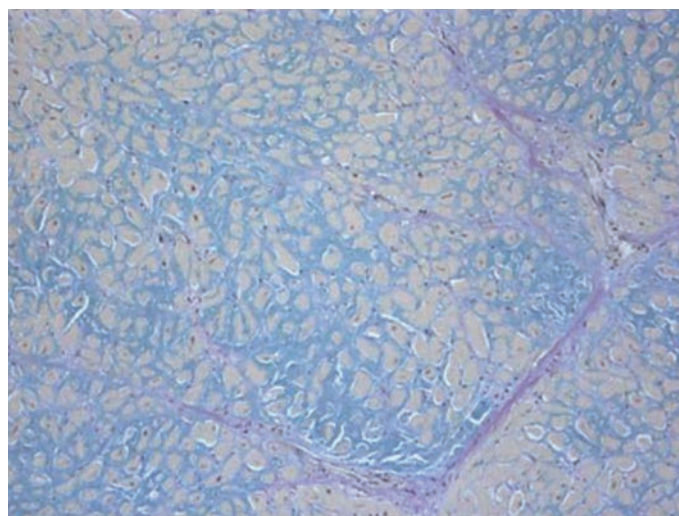
The supportive care of patients with cardiac amyloidosis can be strikingly different from that of ischemic or valvular heart disease. There is no evidence that afterload reduction with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers benefits patients with relaxation abnormalities, and dyspnea and reduced exercise tolerance frequently increase in patients treated with these medications. Fatigue and dyspnea on exertion can also be exacerbated when  $\beta$  blockers are used for rate or rhythm control.

In addition to standard echocardiography, accurate diagnosis of cardiac amyloid requires Doppler flow studies to demonstrate the rapid decline in velocity of blood inflow into the ventricular chambers and optimally conducted cardiac strain studies that demonstrate a decline in the rate of fractional shortening of the ventricular chamber. Patients with unexplained fatigue and/or dyspnea on exertion should have immunofixation of the serum and urine and free light-chain testing to assess for possible light-chain amyloid. However, even if light-chain testing is negative, age-related amyloidosis may still be present because of the deposition of wild-type TTR in the heart.<sup>34</sup> This typically occurs in patients older than age 60 years, predominantly men, half of whom will also have carpal tunnel syndrome.<sup>35,36</sup> Wild-type TTR amyloidosis will not be detected by screening for serum and urine light





A



B

**Figure 19-5.** **A.** Polarized micrograph of Congo red–stained endomyocardial biopsy demonstrates birefringence in the involved area. **B.** Light micrograph of hematoxylin-and-eosin–stained slide of the same specimen demonstrating amyloid as extracellular eosinophilic amorphous material.

chains, and subcutaneous fat aspiration has a lower sensitivity than in AL amyloidosis. These patients require recognition by echocardiography, magnetic resonance imaging (MRI), or endomyocardial biopsy to establish a diagnosis (Fig. 19-5).

Early diagnosis of cardiac amyloid is imperative because it is this group that is responsible for the majority of the early deaths with this disorder. In our experience, 40 percent of newly diagnosed patients with AL amyloidosis will succumb to the disease within the first year, and this percentage has not changed over a quarter century.

Echocardiography remains the most useful test for the imaging and diagnosis of amyloid; however, it is not particularly useful for serial monitoring of patients for response or progression after treatment. The technique still suffers from interobserver variability, and the calculations of the septal thickness can vary substantially on serial measurements. Conversely, a septal thickness greater than 15 mm would be rare with hypertensive cardiomyopathy and would be limited to either amyloidosis or hypertrophic cardiomyopathy. Interestingly, the degree of cardiac infiltration in senile systemic and familial amyloid cardiomyopathy is substantially greater than that seen in AL amyloidosis. A patient with AL amyloidosis with a septal thickness greater than 18 mm will generally have significant disability related to cardiac failure. Patients with TTR amyloidosis, both mutant and wild type, will frequently have septal thicknesses in the range of 25 mm with minimal symptomatology. The old echocardiographic finding of granular sparkling appearance is little used today. Other echocardiographic clues include thickening of the right ventricle and reduction in left ventricular chamber size.<sup>37</sup> Late consequences of cardiac involvement include valvular thickening and valvular regurgitation. Repair of the valve will not result in meaningful improvement in the patient's aerobic exercise capacity. The restriction to flow seen in restrictive cardiomyopathy can be confused with restrictive pericardial disease; occasionally, patients have undergone pericardiectomies without benefit.<sup>38</sup> Endomyocardial biopsy is highly sensitive in the diagnosis of cardiac amyloidosis, and if five specimens are taken, recognition of the diagnosis is virtually certain. Patients with cardiac amyloidosis have poor atrial function and a high incidence of atrial standstill. Atrial and atrial appendage thrombi are well recognized and are potential sources of cardiac embolism.<sup>39</sup> Rare patients can develop amyloid deposition in the coronary microcirculation, resulting in true

ischemic symptomatology and angina with normal epicardial coronary artery anatomy.<sup>40</sup>

Sudden death remains a problem in patients with amyloidosis.<sup>41</sup> The placement of an implantable defibrillator may not reduce the risk of sudden death.<sup>42</sup> Electromechanical dissociation is a common occurrence, and the ability of a fibrillating amyloid heart to be appropriately shocked internally and establish hemodynamic stability has not been established.<sup>43</sup>

Diuretic therapy remains the mainstay of management for cardiac amyloidosis, but in these patients with noncompliant ventricular chambers, higher-than-normal filling pressures are often required to open the ventricle and fill it with blood. Aggressive diuretic therapy will often reduce preload, and this can result in drops in systolic blood pressure, reduced renal blood flow, and syncope.<sup>44</sup>

Familial amyloid cardiomyopathy is rare, but one special TTR mutant known as TTRVal122Ile requires awareness. In a prospective study of cord blood samples, this mutation was found in 3 percent of newborns of American parents of African descent. This compared with a prevalence of 0.44 percent in Americans of European descent and 0 percent of Americans of Hispanic descent. The degree of penetrance of this mutation at the clinical level has not been determined; however, in view of the high incidence and prevalence of this genetic abnormality, the diagnosis of cardiac amyloidosis in Americans of African descent warrants early analysis of the DNA for this mutation.<sup>45,46</sup>

Cardiac biomarkers play an important role in the prognosis of amyloid as well as in its functional assessment.<sup>47</sup> Both the B-natriuretic peptide and troponin levels predict outcomes in patients with amyloidosis and are important parts of the staging system for this disease.<sup>48</sup> Staging involves assigning a point for any of the following characteristics: difference between the involved and uninvolved free light chain of greater than 180 mg/L; a cardiac troponin T level greater than 0.025 ng/mL; and the N-terminal of the pro-hormone brain natriuretic peptide (NT-proBNP) level greater than 1800 pg/mL. This creates four stages with median survivals ranging from 6 months (stage 4) to 60 months (stage 1). Serialized measurements of the NT-proBNP also have been used to define response and progression and, in fact, have supplanted the use of serial echocardiography to assess changes over time, both in following the natural

**TABLE 19-3.** Suggested Testing of a Known Amyloid Patient

|                                                                                                         |
|---------------------------------------------------------------------------------------------------------|
| If mass spectroscopy identifies light-chain amyloid:                                                    |
| Consider localized amyloidosis (bladder, larynx, skin, bronchi)                                         |
| If systemic (visceral involvement), perform the following tests:                                        |
| Alkaline phosphatase                                                                                    |
| Aspartate aminotransferase                                                                              |
| $\beta_2$ -Microglobulin                                                                                |
| Bilirubin                                                                                               |
| Calcium                                                                                                 |
| Creatinine                                                                                              |
| Glucose                                                                                                 |
| Complete blood count                                                                                    |
| Immunoglobulin free light chains                                                                        |
| Immunofixation and electrophoresis                                                                      |
| Serum and 24-hour urine                                                                                 |
| Quantitative immunoglobulins                                                                            |
| N-terminal probrain natriuretic peptide                                                                 |
| Troponin T                                                                                              |
| Factor X level                                                                                          |
| Chest x-ray                                                                                             |
| Electrocardiogram                                                                                       |
| Echocardiogram                                                                                          |
| Doppler and strain imaging                                                                              |
| Creatinine clearance                                                                                    |
| If mass spectroscopy identifies transthyretin (TTR) amyloid, perform these tests:                       |
| Echocardiogram                                                                                          |
| Doppler and strain imaging                                                                              |
| Familial amyloidosis genetic testing (mass spectroscopy of serum TTR; if abnormal, TTR gene sequencing) |

history and in assessing response to therapeutic interventions.<sup>49–51</sup> All patients who present with amyloidosis, whether or not cardiac disease is suspected, should have cardiac biomarker measurements performed. Table 19–3 lists the suggested diagnostic tests to perform when a patient with amyloidosis is seen.

Contrast-enhanced cardiac MRI is increasingly being used in the assessment of cardiac amyloid. Cardiac MRI (Fig. 19–6) can measure the thickness of the myocardium accurately and, after gadolinium injection, can demonstrate delayed subendocardial enhancement that can be quite specific for cardiac amyloidosis.<sup>52,53</sup> Phase-contrast MRI provides information on flow dynamics, diastolic filling parameters, and mitral peak in-flow velocity.<sup>54</sup> By providing a functional assessment, this technique can help establish an early diagnosis and has the potential to assess response to therapy. MRI cannot be used in the presence of a pacemaker or defibrillator or if there is renal insufficiency because gadolinium is contraindicated. Radionuclide scanning is being explored for amyloidosis. Technetium pyrophosphate is a sensitive marker for the presence of ATTR amyloidosis, both wild type and mutant, whereas uptake is not seen in AL amyloidosis.<sup>55</sup> A recent small study assessing <sup>11</sup>C-labeled Pittsburgh Compound-B (<sup>11</sup>C-PiB)-based positron emission tomographic (PET) imaging identified cardiac amyloid deposits in all patients with light-chain amyloidosis and ATTR and was negative in controls.<sup>56</sup> Therefore, nuclear imaging in the future may not only help

confirm cardiac involvement by amyloidosis, but also help differentiate one type versus another.

### Liver

Liver infiltration from amyloid can be seen in up to one-quarter of patients with AL amyloidosis.<sup>17</sup> These patients will present with hepatomegaly, elevation of the serum alkaline phosphatase, with normal or near-normal transaminases and bilirubin. Half of the patients with hepatic amyloidosis have renal amyloidosis, which dominates the clinical syndrome. When a patient presents with hepatomegaly and imaging shows no filling defects, the presence of proteinuria, a monoclonal protein, or the presence of Howell-Jolly bodies in a blood film indicative of hyposplenism are highly suggestive of amyloidosis. Most patients have symptoms consistent with chronic liver disease, early satiety, anorexia, and unexplained weight loss. It is common to find spider telangiectasias on the upper chest. Portal hypertension and ascites are uncommon, however.<sup>57</sup> Rare instances of both hepatic and splenic rupture have been reported.<sup>58</sup> The diagnosis can usually be established with fat aspiration and marrow biopsy, but a liver biopsy is safe and does not have a higher risk of bleeding complications than in other patients with liver disease.

### Nervous System

Amyloid, by virtue of its deposition in the vasa nervorum, causes a mixed axonal and demyelinating peripheral neuropathy. The neuropathy is symmetric, tends to ascend beginning in the toes, and eventually involves the upper extremities. It causes paresthesias and often painful dysesthesias and eventually causes motor loss. Approximately half of the patients have an associated carpal tunnel syndrome, and approximately one-quarter have associated autonomic features, including orthostatic hypotension; autonomic dysmotility of the gastrointestinal tract, including vomiting because of pseudoobstruction and alternating constipation with diarrhea, often intractable; and bladder abnormalities, including overflow incontinence and incomplete emptying.<sup>59,60</sup> The progression of amyloid neuropathy is slow, and it is common to have delays of 2 to 3 years before a diagnosis is established. Electromyography is not particularly useful in the early diagnosis because amyloid preferentially affects the small unmyelinated fibers of the extremities, which are not well assessed with standard electrodiagnostic studies. Patients with an unexplained peripheral neuropathy who are not diabetic should have immunofixation of serum and urine and a free light-chain assay performed. A positive finding requires the consideration of amyloidosis in the differential, although, for patients who have an IgM monoclonal protein, the possibility that the IgM is directly responsible for neuropathy in the absence of amyloid is significant. Sural nerve biopsies can usually detect the amyloid deposits, but there are reports in the literature of sural nerve biopsies having missed proven amyloidosis.<sup>61</sup> Mass spectroscopic analysis of the amyloid deposits is particularly important because of the high prevalence of neuropathy in patients with non-AL amyloidosis.

## THERAPY

Treatment of amyloidosis has improved significantly with the introduction of novel agents and refinements in autologous stem cell transplantation. For years, the regimen of melphalan and prednisone was standard therapy, but the response rate never exceeded 25 percent, and the impact on survival was unimpressive. Melphalan and prednisone therapy has now been supplanted by the use of melphalan and dexamethasone, which has a very low therapy-related mortality and can be administered to patients with cardiac and renal failure as well as frail patients. The 5-year actuarial survival in patients treated with

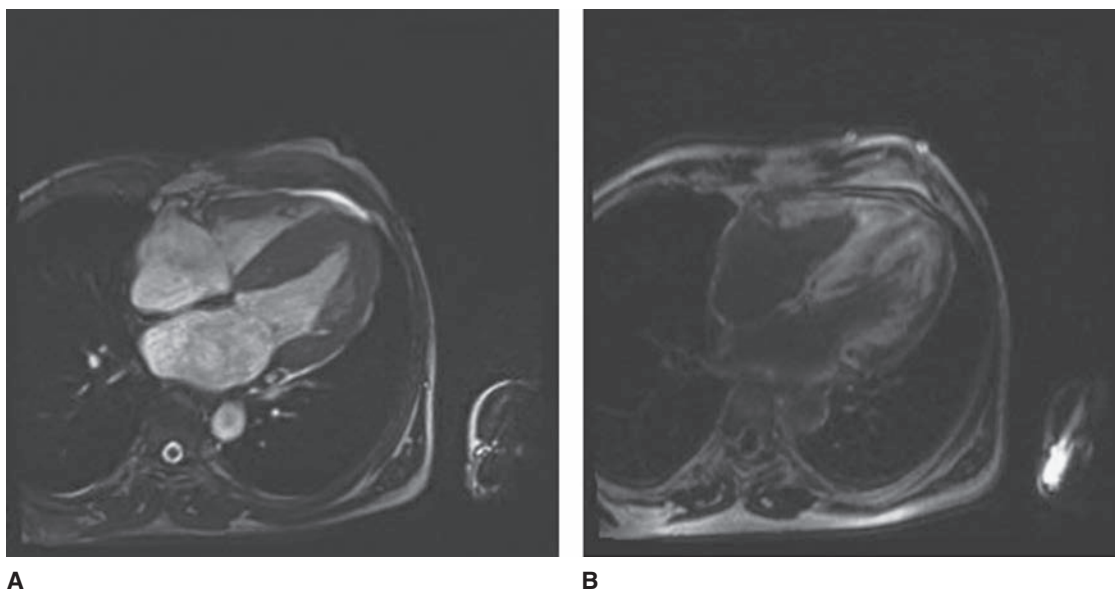


**Figure 19-6.** **A.** Magnetic resonance imaging demonstrating thickened interventricular septum from amyloid infiltration. **B.** Delayed gadolinium enhancement on subendocardial tissue is characteristic of amyloid cardiomyopathy.

melphalan and dexamethasone is 50 percent but is highly dependent on the enrolled population. Median responses as short as 10.5 and 17.5 months have been reported and are a function of the proportion of patients with advanced cardiac amyloid enrolled. Re-treatment with melphalan and dexamethasone after an initial response also can be successful. It is often difficult to interpret results in phase II trials because of patient heterogeneity. Stratification of patients, based on the staging mentioned previously, is important in reporting outcomes. Melphalan and dexamethasone should be considered one option in patients who are not eligible for stem cell transplantation.<sup>62</sup>

### AUTOLOGOUS STEM CELL TRANSPLANT

Most experts believe that autologous stem cell transplantation is the treatment of choice for patients with amyloidosis who do not have severe end-organ dysfunction, even though this approach has not been proven superior to conventional chemotherapy in prospective randomized trials or meta-analyses. However, it must be recognized that no more than 30 percent of patients with amyloidosis are eligible for stem cell transplantation. Currently, the therapy-related mortality since 2010 at the Mayo Clinic is 0.5 percent (six out of 285), and the 10-year overall survival is 43 percent. Significant improvements in organ function and quality of life are commonly observed following stem cell transplantation and are strongly correlated with attainment of a very good partial response or better.<sup>63–65</sup> To reduce treatment-related mortality, current patient selection for transplantation includes age younger than 70 years, serum creatinine less than 1.8 mg/dL, serum troponin T less than 0.06, and an NT-proBNP less than 8500 pg/mL. Systolic blood pressure greater than 90 mmHg is an important criterion for safe transplantation. The median age at the time of transplantation at the Mayo Clinic is 57 years. The median percentage plasma cells in the marrow of transplanted patients is 7 percent, and the median urinary protein loss is just under 4 g/day. Seventy percent of patients who receive autologous stem cell transplantation have predominant renal involvement, 12 percent have peripheral neuropathy, and 14 percent have liver involvement. Patients with advanced cardiac involvement are generally excluded from transplantation, although half of transplanted patients have mild-to-moderate cardiac involvement as assessed by biomarkers and echocardiogram.

Blood stem cells are generally mobilized using leukocyte growth factors alone without cytotoxic chemotherapy to avoid hemodynamic deterioration during the mobilization process. A median of two aphereses is required to collect adequate stem cells for transplantation. The standard conditioning regimen is melphalan 200 mg/m<sup>2</sup>, although the dose may be reduced for multiorgan involvement, age, and frailty. Induction chemotherapy is not generally administered prior to autologous stem cell transplantation, although this approach is considered in patients with over 10 percent marrow plasmacytosis because of data suggesting an inferior outcome in such patients. In 2013, 40 percent of patients were transplanted at the Mayo Clinic without requiring hospitalization, and of the 60 percent of patients who were hospitalized, the median hospital stay was 6 days. Leukocyte growth factors are not routinely used after transplantation because they increase fluid retention. A partial response or better is seen in 75 percent of patients. Complete hematologic responses are documented in 40 percent of patients. Organ responses are seen in 50 percent of patients transplanted at the Mayo Clinic. Median survival for complete responders has not been reached, whereas patients with partial response or better have a median survival of 107 months. Predictors of outcome following autologous stem cell transplantation include cardiac biomarkers, NT-proBNP, and troponin.<sup>66</sup>

### IMMUNOMODULATORY DRUGS

The combination of melphalan, dexamethasone, and lenalidomide has been reported for the treatment of AL amyloidosis. The maximum tolerated dose of lenalidomide was found to be 15 mg. Melphalan doses lower than those recommended for myeloma patients are used (0.18 mg/kg/day for 4 days every 28 days), with a complete response rate of 42 percent, an overall response rate of 58 percent, and a 2-year overall survival of 81 percent. Prophylaxis against deep venous thrombosis is required when lenalidomide is used; physicians at the Mayo Clinic prefer use of aspirin, 325 mg, orally, daily. A combination of cyclophosphamide, thalidomide, and dexamethasone is safe and effective. In a risk-adapted, all-oral therapy trial, 75 patients (with performance status of 0–1) were treated, with a hematologic response in 74 percent, of which 21 percent were complete. The 3-year estimated overall survival was 82 percent,



and grade 2 toxicity was seen in 52 percent. Lenalidomide and dexamethasone have been used in amyloidosis in a fashion identical to that used for multiple myeloma. It is important to remember that lenalidomide can increase the level of NT-proBNP and may actually aggravate heart failure.<sup>67</sup> In addition, the typical dose of lenalidomide used to treat multiple myeloma is not well tolerated by AL patients and treatment should be initiated at a dose of no more than 15 mg daily. There is a high discontinuation rate of lenalidomide within the first three cycles. Myelosuppression, skin rash, and fatigue are common. In a study of lenalidomide and dexamethasone in patients who had failed melphalan and bortezomib, two patients died prior to first-response evaluation, and 50 percent experienced grade 3 or greater toxicity. The hematologic response rate was 41 percent. The median overall survival was 14 months. Cyclophosphamide, lenalidomide, and dexamethasone have been used in the treatment of amyloidosis, with lenalidomide at 15 mg per day and cyclophosphamide at 100 mg per day. A partial response or greater was observed in 55 percent of patients, with complete responses in 8 percent, organ responses in 40 percent, and a 2-year overall survival of 41 percent. Cyclophosphamide, lenalidomide, and dexamethasone hematologic response rates ranged from 40 to 77 percent in three different studies. Complete responses did not exceed 10 percent. Lenalidomide toxicity was substantial and included fatigue and fluid retention. At the Mayo Clinic, we use lenalidomide as second-line therapy for non-transplant-eligible patients, preferably with no or minimal cardiac involvement.

Twenty-eight patients received pomalidomide 2–4 mg/day with no dose-limiting toxicity. Pomalidomide was administered continuously, and dexamethasone was given once per week at a dose of 20 or 40 mg. Fifteen patients experienced grade 3 to 4 adverse events; the most common were fluid retention and infection. Hematologic response was observed in 68 percent of patients (very good partial response or complete response in 29 percent), as well as improved survival. Median time to response was 1 month.<sup>68</sup>

Pomalidomide has been used with dexamethasone in relapsed amyloidosis. In a population of 33 evaluable patients, the response rate was 48 percent with a median time to response of 2 months. Organ improvement was found in 15 percent. The progression-free survival was 14 months; overall survival was 28 months.<sup>69</sup>

Bortezomib is highly active in the treatment of amyloidosis.<sup>70</sup> The combination with dexamethasone in untreated patients produces a 47 percent complete response rate, with higher responses seen in patients treated with twice-weekly bortezomib. The cardiac response rate is 29 percent. Hematologic responses are associated with cardiac response as measured by a reduction in NT-proBNP. Bortezomib also has been used with melphalan and dexamethasone, as well as cyclophosphamide with dexamethasone.<sup>71</sup> The CyBORd (cyclophosphamide, bortezomib, and dexamethasone) regimen was reported by the Mayo Clinic as a retrospective review of 17 patients, with responses seen in 94 percent (71 percent complete, 24 percent partial) and a median time to response of 2 months.<sup>72</sup> In many published trials, patients with severe cardiac involvement were excluded; as a consequence, it is difficult to determine the efficacy of bortezomib in this difficult subgroup.<sup>73</sup> In one study of 38 patients with advanced cardiac disease using reduced doses of twice-weekly bortezomib and dexamethasone, 18 of 38 patients died during therapy; 21 patients achieved a hematologic response after a median of three cycles. In a series of 35 patients treated with bortezomib-dexamethasone with subsequent addition of cyclophosphamide, 86 percent achieved a rapid response. A second trial of cyclophosphamide-bortezomib-dexamethasone resulted in hematologic responses in 68 percent and a 1-year overall survival of 65 percent. Because bortezomib has been reported to increase the severity of heart failure in myeloma, it is clearly a concern that requires monitoring in patients. The oral proteasome inhibitor MLN9708, ixazomib, is currently under

investigation. In a retrospective review of 234 patients treated with bortezomib-dexamethasone or high-dose melphalan, 20 were treated with the former combination and 30 received high-dose melphalan. The bortezomib-dexamethasone group showed a trend of lower treatment-related mortality (5.0 vs. 10.0 percent,  $p = 0.641$ ), greater hematological response (partial response or better) (90.0 vs. 73.3 percent,  $p = 0.279$ ), higher complete response (60 vs. 50 percent,  $p = 0.487$ ), and similar survival with the high-dose melphalan group (neither reached,  $p = 0.705$ ). Bortezomib-dexamethasone was as effective and safe as high-dose melphalan.<sup>74</sup> Bortezomib and dexamethasone have been used as consolidation therapy following stem cell transplantation.<sup>75</sup> Forty patients were transplanted with risk-adapted melphalan; patients with less than a complete response received consolidation with bortezomib-dexamethasone. Survivals at 12 and 24 months posttreatment were 88 and 82 percent, respectively. Of the original 40 patients, 23 received consolidation, and the response was enhanced in 20 (86 percent). Organ response occurred in 70 percent at 24 months. The use of bortezomib-dexamethasone consolidation following stem cell transplantation can help deepen the response in patients who do not achieve a complete response.

## PROTEASOME INHIBITORS

Ixazomib is a second-generation proteasome inhibitor. It is given orally, once weekly for 3 weeks of a 4-week cycle, at a recommended dose of 4 mg weekly. The drug was evaluated in 22 AL patients with a median of three prior therapies. Sixteen patients had received prior bortezomib, and 6 patients were bortezomib naïve. Toxicity was manageable, and there were no reports of cardiorespiratory events. Deep hematological responses ( $\geq$  very good partial remission) occurred in 43 percent of patients and were associated with a cardiac response and longer progression-free survival. An international phase III randomized study is under way assessing ixazomib plus dexamethasone compared to physician's choice of five options in patients with relapsed or refractory AL amyloidosis not previously exposed to proteasome inhibitors.<sup>76</sup>

## MONOCLONAL ANTIBODIES

Three new monoclonal antibodies are in development targeting the dissolution of amyloid deposits in tissues. An antibody targeting SAP, a glycoprotein in all amyloid fibrils, has been reported to effectively dissolve amyloid deposits, confirmed by SAP scanning demonstrating resolution of liver and spleen deposits. The antibody, 11-F4, has single-agent activity. Another antibody, NEO-D1, has been studied in a phase I–II trial, and both renal and cardiac biomarker responses have been reported. Antibodies combined with chemotherapy are both in phase IIb and phase III registration trials in patients with cardiac amyloidosis. The prospect of treating light-chain synthesis as well as resident fibrils in tissues offers the potential for improved outcomes.

### Daratumumab

Daratumumab is a humanized IgG-K monoclonal antibody that targets CD38, a surface marker found on plasma cells. It has single-agent activity in relapsed or refractory myeloma and a synergistic effect in combination with a proteasome inhibitor or with an IMiD. Its toxicity profile is low, mainly infusion reactions at initial administration. The first report on daratumumab use in AL amyloidosis included two myeloma patients who developed extensive AL amyloidosis during the course of their disease.<sup>77</sup> Daratumumab was infused at the prescribed dose and schedule for myeloma and was able to achieve rapid normalization of the involved light chain. Treatment was well tolerated without significant toxicities. Two additional series have been reported, one with 18 and the other with 7 patients. In the first report, daratumumab

yielded a response in 88 percent of patients, including deep response ( $\geq$  very good partial remission or better) in 41 percent of patients treated. In a subset of 12 patients with amyloid cardiomyopathy in that study, 83 percent achieved a response (60 percent very good partial to complete remission), and some also crossed the threshold for cardiac response.<sup>78</sup>

In a second report, in which all patients had prior autologous hematopoietic stem cell transplantation, a hematological response occurred in all patients (71 percent had a substantial response). Infusion reactions occurred in most patients, but were largely mild. These data indicate that daratumumab will have a role in the management of AL amyloidosis.

## COURSE AND PROGNOSIS

In spite of the introduction of new agents, late diagnosis, particularly of cardiac amyloid, remains a major barrier to improvement in the overall survival of patients; despite recent improvements in survival, 40 percent of patients succumb to the disease within the first year after diagnosis. This early mortality has not changed in 30 years. Patients can have a hematologic response and still die of end-organ damage because it is impossible to repair the tissue damage that has occurred prior to diagnosis.<sup>79,80</sup> The prognosis in amyloidosis is determined by two primary factors. The first is the extent of cardiac involvement, and the second is the plasma cell burden seen in these patients. The former, historically measured by echocardiography, is more reproducibly measured by the use of cardiac biomarkers. The percentage of plasma cells in the marrow has an important impact on prognosis.<sup>10,81</sup> The best surrogate and the most reproducible way to measure the plasma cell burden is to look at the difference between the involved and uninvolved light chains (dFLC) in the serum. A four-stage prognostic model has been developed using measurements of NT-proBNP, troponin, and serum-free light chains. One point each is assigned for a troponin T level of 0.025 ng/mL or greater, an NT-proBNP greater than 1800 pg/mL, and a free light chain difference of greater than 180 mg/L. This provides almost four equal-size groups (if you have none of the three, it is stage 1; if you have one, it is stage 2; if you have two, it is stage 3; and if you have all three, it is stage 4) with median overall survivals of 94, 40, 14, and 6 months, respectively. These serum tests are currently the standard for assessing prognosis in amyloidosis. Assessing response in amyloidosis is a twofold process. First, the hematologic response is assessed by determining the reduction of the plasma cell burden and of the production of precursor amyloid light chains achieved by systemic therapy. There are four classes of response: (1) complete remission, defined by negative immunofixation of serum and urine and a normal immunoglobulin free light-chain ratio; (2) a very good partial response that requires the difference between involved and uninvolved serum-free light chain be less than 40 mg/L; (3) a partial response, defined as a 50 percent decrease from baseline in the dFLC; and (4) failure to respond, including all other patients. Light-chain assessment not only is the ideal method for measuring response because of the rapid decline in levels if therapy is effective, but also is better than the intact immunoglobulin as a measure of response when both are present.<sup>82</sup> Although hematologic response is the first goal, the purpose of therapy is preservation of organ function. Therefore, to have a meaningful impact, an organ response should be seen. Current data indicate that organ response rates are directly linked to hematologic response rates; the deeper the hematologic response, the more likely there will be an organ response. A renal response is defined as a 50 percent decrease in 24-hour urine protein. The decrease must be no less than 0.5 g/day without a change in serum creatinine. A 50 percent increase in urinary protein loss to at least 1 g/day or a 25 percent worsening of creatinine clearance is indicative of progression. Cardiac response is defined

primarily by changes in the NT-proBNP. A 30 percent reduction in the NT-proBNP, a minimum of 300 ng/L in patients whose baseline NT-proBNP is greater than 650 ng/L, constitutes a response. However, we have seen patients in whom fluctuations of the NT-proBNP of as much as 30 percent can occur as a result of changes in diuretics or the development of superimposed pulmonary infections that are unrelated to true changes in cardiac function; consequently, some caution is warranted in interpreting the results. An improvement in New York Heart Association class by two stages from 4 to 2 or 3 to 1 also is considered a response. Historically, echocardiography has been used to assess response, but interobserver variability has rendered it less useful, and echocardiography is not currently part of response criteria. Cardiac progression constitutes a 30 percent increase in NT-proBNP, a minimum increase of 300 ng/dL. Finally, liver response is defined as a 50 percent reduction in the abnormal alkaline phosphatase value, with progression defined as a 50 percent increase of alkaline phosphatase above the lowest recorded value. Currently, consensus criteria to define response in soft tissues, the gastrointestinal tract, the tongue, the lung, or the peripheral nerve do not exist for AL amyloidosis.

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## CHAPTER 20

# MACROGLOBULINEMIA

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### SUMMARY

Waldenström macroglobulinemia (WM) is an indolent B-cell neoplasm manifested by the accumulation of clonal immunoglobulin (Ig) M secreting lymphoplasmacytic cells. MYD88<sup>L265P</sup> and CXCR4 WHIM (warts, hypogammaglobulinemia, infections, myelokathexis)–like somatic mutations are present in more than 90 percent and 30 to 35 percent of WM patients, respectively, and impact disease presentation, treatment outcome, and/or overall survival. Familial predisposition is common in WM. Asymptomatic patients should be observed. Patients with disease-related hemoglobin of less than 10 g/dL, platelets less than  $100 \times 10^9/L$ , bulky adenopathy and/or organomegaly, symptomatic hyperviscosity, peripheral neuropathy, amyloidosis, cryoglobulinemia, cold-agglutinin disease, or transformed disease should be considered for therapy. Plasmapheresis should be used for patients with symptomatic hyperviscosity and prerituximab for those with high serum IgM levels to preempt a symptomatic IgM flare. The treatment choice should take into account specific goals of therapy, necessity for rapid disease control, risk of treatment-related neuropathy, immunosuppression and secondary malignancies, and planning for future autologous stem cell transplantation. Frontline treatments include rituximab alone or combined with alkylating agents (bendamustine, cyclophosphamide); proteasome inhibitors (bortezomib, carfilzomib); nucleoside analogues (fludarabine, cladribine); and ibrutinib. In case of relapsed or treatment-resistant patients, an alternative frontline regimen or autologous stem cell transplantation can be considered. Everolimus can be considered for those patients with multiple relapses.

### DEFINITION AND HISTORY

Waldenström macroglobulinemia (WM) is a lymphoid neoplasm resulting from the accumulation, predominantly in the marrow, of a clonal population of lymphocytes, lymphoplasmacytic cells, and plasma cells, which secrete a monoclonal immunoglobulin (Ig) M.<sup>1</sup> WM corresponds to lymphoplasmacytic lymphoma (LPL) as defined in the Revised European-American Lymphoma (REAL) and World Health Organization (WHO) classification systems.<sup>2,3</sup> Most cases of LPL are WM; less than 5 percent of cases are IgA-secreting, IgG-secreting, or nonsecreting LPL.

**Acronyms and Abbreviations:** CD16, FcγRIIIa receptor; CD40L, CD40 ligand; CDR, complement determination region; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; GM<sub>1</sub>, ganglioside M<sub>1</sub>; HCV, hepatitis C virus; Ig, immunoglobulin; IL, interleukin; κ, kappa light chain; LPL, lymphoplasmacytic lymphoma; MAG, myelin-associated glycoprotein; R-CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone, and rituximab; R-CP, cyclophosphamide, prednisone, and rituximab; R-CVP, cyclophosphamide, vincristine, prednisone, and rituximab; sCD27, soluble CD27; WHO, World Health Organization; WM, Waldenström macroglobulinemia.

In 1944, Jan Waldenström, a Swedish physician-scientist, reported in *Acta Medica Scandinavica* three cases of a disease he presciently thought was related to myeloma but for the absence of bone involvement and the scarcity of plasma cells in the infiltrate of small lymphocytes. He noted the increase in plasma protein concentration, marked increased serum viscosity, exaggerated bleeding and retinal hemorrhages, and virtually every other feature of the disorder in his case descriptions. In collaboration with a colleague, he showed, using ultracentrifugation and electrophoresis, that the abundant abnormal protein had a molecular weight of approximately 1 million and was not an aggregate of smaller proteins. The disease, which he described with such thoroughness, was later named in his honor.

### EPIDEMIOLOGY

The age-adjusted incidence rate of WM is 3.4 per 1 million among males and 1.7 per 1 million among females in the United States. It increases in incidence geometrically with age.<sup>4,5</sup> The incidence rate is higher among Americans of European descent. Americans of African descent represent approximately 5 percent of all patients.

Genetic factors play a role in the pathogenesis of WM. Approximately 20 percent of WM patients are of Ashkenazi-Jewish ethnic background.<sup>6</sup> Familial disease has been reported commonly, including multigenerational clustering of WM and other B-cell lymphoproliferative diseases.<sup>7–10</sup> Approximately 25 percent of 924 sequential patients with WM presenting to a tertiary referral center had a first-degree or second-degree relative with either WM or another B-cell disorder. Familial clustering of WM with other immunologic disorders, including hypogammaglobulinemia and hypergammaglobulinemia (particularly polyclonal IgM), autoantibody production (particularly to the thyroid), and manifestation of hyperactive B cells has also been reported in relatives without WM.<sup>10</sup> Increased expression of the *BCL-2* gene with enhanced survival has been observed in B cells from familial patients and their family members.<sup>10</sup>

The role of environmental factors is uncertain, but chronic antigenic stimulation from infections and certain drug or chemical exposures have been considered but have not reached a level of scientific certainty. Hepatitis C virus (HCV) infection was implicated in WM causality in some series, but in a study of 100 consecutive WM patients in whom serologic and molecular diagnostic studies for HCV infection were performed, no association was found.<sup>11–13</sup>

### PATHOGENESIS

#### NATURE OF THE WALDENSTRÖM MACROGLOBULINEMIA CLONE

Examination of the B-cell clone(s) found in the marrow of WM patients reveals a range of differentiation, from small lymphocytes with large focal deposits of surface immunoglobulins, to lymphoplasmacytic cells, to mature plasma cells that contain intracytoplasmic IgM (Fig. 20–1).<sup>14</sup> Circulating clonal B cells are often detectable in patients with WM, although lymphocytosis is uncommon.<sup>15,16</sup> WM cells express the monoclonal IgM, and some clonal cells also express surface IgD.<sup>17</sup> The characteristic immunophenotypic profile of WM lymphoplasmacytic cells includes the expression of the pan-B-cell markers CD19, CD20 (including FMC7), CD22, and CD79.<sup>17,18</sup> Expression of CD5, CD10, and CD23 can be present in 10 to 20 percent of cases, and their presence does not exclude the diagnosis of WM.<sup>19</sup> Multiparameter flow cytometric analysis has also identified CD25 and CD27 as being characteristic of the WM clone and found that a CD22<sup>dim</sup>/CD25<sup>+</sup>/CD27<sup>+</sup>/IgM<sup>+</sup> population may be present among clonal B lymphocytes in patients with essential

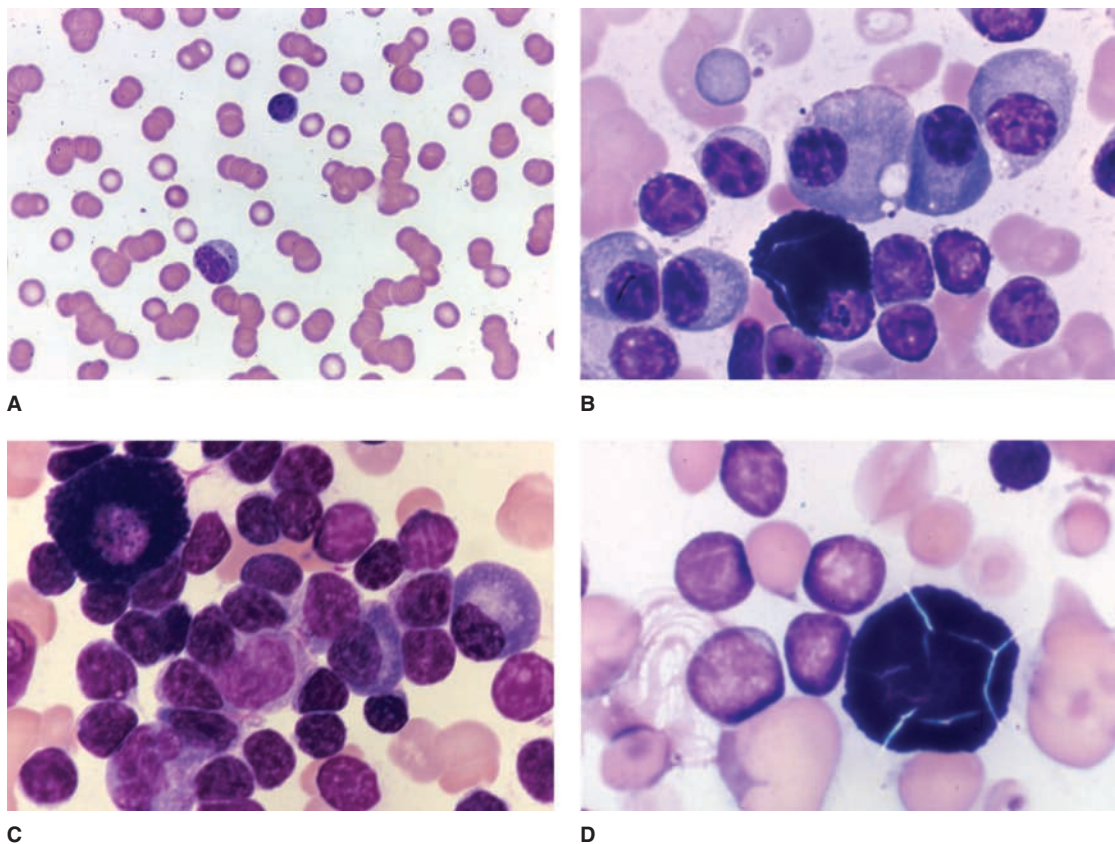


**Figure 20-1.** Waldenström macroglobulinemia. **A.** Blood film displaying the characteristic pathologic rouleaux seen as a result of the red cell aggregating properties of immunoglobulin M. **B.** Marrow film showing characteristic infiltrate of lymphocytes, lymphoplasmacytic cells, and plasma cells. A mast cell is evident lower center. Although not specific for this disease, mast cells are commonly present in the marrow. **C.** Marrow film showing infiltrate of lymphocytes with occasional plasma cells and a mast cell. **D.** Marrow film showing lymphocytic infiltrate with a “cracked” mast cell sometimes seen in this disease. The fraction of plasma cells varies, as shown by the somewhat higher proportion in (**B**) as compared to (**C**) and (**D**). Lymphocytes and lymphoplasmacytic cells predominate. (Reproduced with permission from Lichtman’s Atlas of Hematology, [www.accessmedicine.com](http://www.accessmedicine.com).)

monoclonal gammopathy (synonym: monoclonal gammopathy of unknown significance [MGUS]) of the IgM type who ultimately progressed to WM.<sup>20</sup>

Somatic mutations in immunoglobulin genes are present with an increased frequency of nonsynonymous as compared to silent mutations in complement-determining regions, along with somatic hypermutation, thereby supporting a postgerminal center derivation for the WM B-cell clone in most patients.<sup>21,22</sup> A strong preferential usage of *VH3/JH4* gene families without intraclonal variation, and without evidence for any isotype-switched transcripts, is present.<sup>23,24</sup> These data support an IgM<sup>+</sup> and/or IgM<sup>+</sup>IgD<sup>+</sup> memory B-cell origin for most cases of WM.

In contrast to myeloma plasma cells, no recurrent translocations have been described in WM, which can help to distinguish WM from IgM myeloma cases, as IgM myeloma cases often exhibit t(11;14) translocations.<sup>25,26</sup> Despite the absence of IgH translocations, recurrent chromosomal abnormalities are present in WM cells. These include deletions in chromosome 6q21–23 in 40 to 60 percent of WM patients, with concordant gains in 6p in 41 percent of 6q-deleted patients.<sup>27–30</sup> In a series of 174 untreated WM patients, 6q deletions, followed by trisomy 18, 13q deletions, 17p deletions, trisomy 4, and 11q deletions, were observed.<sup>30</sup> Deletion of 6q and trisomy 4 were associated with adverse prognostic markers in this series. As 6q deletions represent the most recurrent cytogenetic finding in WM cases, there has been interest in identifying the region of minimal deletion and possible target genes within this

region. Two putative gene candidates within this region are *TNFAIP3*, a negative regulator of nuclear factor kappa B (NF- $\kappa$ B) signaling, and *PRDM1*, a master regulator of B-cell differentiation.<sup>29,31</sup> The removal of a NF- $\kappa$ B–negative regulator is of particular interest as the phosphorylation and translocation of NF- $\kappa$ B into the nucleus is a crucial event for WM cell survival.<sup>32</sup> The success of proteasome inhibitor therapy in WM may occur because the degradation of negative regulators of NF- $\kappa$ B, such as the inhibitor of kappa B (I $\kappa$ B), is blocked.<sup>33,34</sup>

## MUTATION IN MYD88

A highly recurrent somatic mutation (MYD88<sup>L265P</sup>) was first identified in WM patients by whole-genome sequencing (WGS) and confirmed by multiple studies through Sanger sequencing and/or allele-specific polymerase chain reaction (PCR) assays.<sup>35–40</sup> MYD88<sup>L265P</sup> is expressed in 90 to 95 percent of WM cases when more sensitive allele-specific PCR has been employed, using both CD19-sorted and unsorted marrow cells.<sup>36–40</sup> By comparison, MYD88<sup>L265P</sup> was absent in myeloma samples, including IgM myeloma, and was expressed in a small subset (6 to 10 percent) of marginal zone lymphoma patients, who surprisingly have WM-related features.<sup>36–38,41</sup> Non-L265P MYD88 mutations are also rarely present in WM.<sup>42</sup> By PCR assays, 50 to 80 percent of IgM MGUS patients also express MYD88<sup>L265P</sup>, and expression of this mutation was associated with increased risk for malignant progression.<sup>36–38,43</sup> The presence of MYD88<sup>L265P</sup> in an IgM MGUS patient suggests a role for



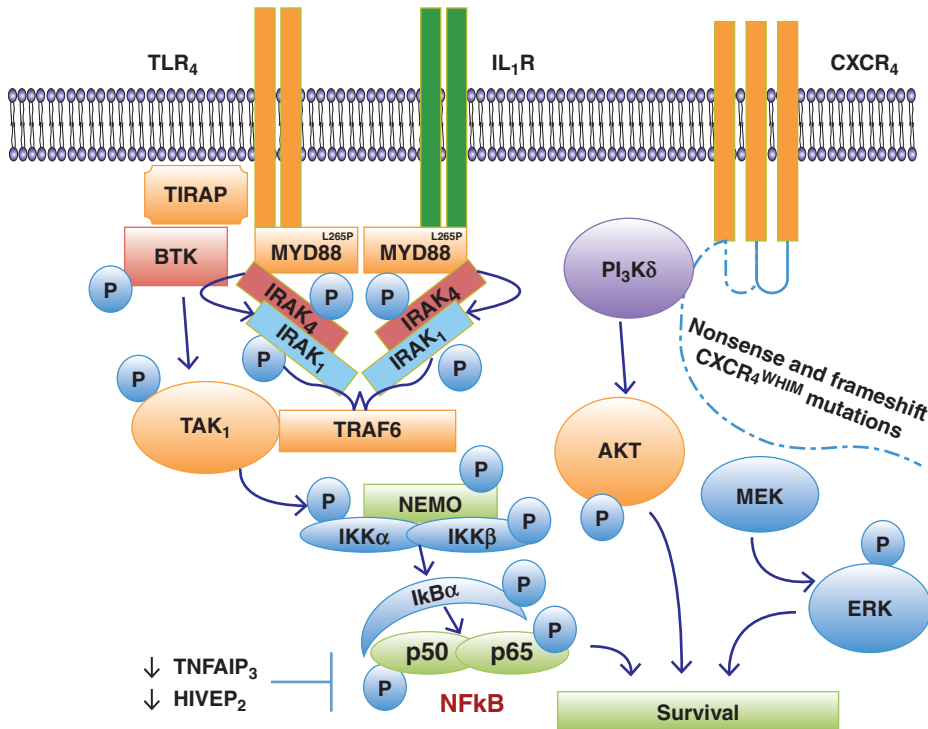


**Figure 20-2.** MYD88<sup>L265P</sup> and CXCR4<sup>WHIM</sup> mutations are highly prevalent in patients with Waldenström macroglobulinemia and trigger transcriptional factors that include nuclear factor kappa B (NF-κB), AKT, and extracellular signal-regulated kinase (ERK) that support the growth and survival of lymphoplasmacytic cells. BTK, Bruton tyrosine kinase; IKK, I kappa kinase; IL-1R, interleukin-1 receptor; IκB, inhibitor of kappa B; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; NEMO, nuclear factor-kappa-B essential modulator; PI3Kδ, phosphoinositide 3-kinase; TIRAP, toll-interleukin 1 receptor (TIR) domain-containing adaptor protein; TRAF, tumor necrosis factor (TNF) receptor-associated factor; TLR, toll-like receptor.

this mutation as an early oncogenic driver, and other mutations and/or copy number alterations leading to abnormal gene expression are likely to promote disease progression.<sup>29</sup>

The impact of MYD88<sup>L265P</sup> on growth and survival signaling in WM cells has been addressed in several studies (Fig. 20-2). Knockdown of MYD88 decreased survival of MYD88<sup>L265P</sup>-expressing WM cells, whereas survival was enhanced by knock-in of MYD88<sup>L265P</sup> versus wild-type (WT) MYD88.<sup>44</sup> The discovery of a mutation in MYD88 is significant given its role as an adaptor molecule in toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling.<sup>45</sup> All TLRs except for TLR3 use MYD88 to facilitate their signaling. Following TLR or IL-1R stimulation, MYD88 is recruited to the activated receptor complex as a homodimer, which then complexes with interleukin 1 receptor associated kinase (IRAK) and activates IRAK1 and IRAK2.<sup>46–48</sup> Tumor necrosis factor receptor-associated factor 6 is then activated by IRAK1, leading to NF-κB activation via IκBα phosphorylation.<sup>49</sup> Use of inhibitors of the MYD88 pathway led to decreased IRAK1 and IκBα phosphorylation, as well as survival of MYD88<sup>L265P</sup>-expressing WM cells. These observations are of particular relevance to WM since NF-κB signaling is important for WM growth and survival.<sup>50</sup> Bruton tyrosine kinase (BTK) is also activated by MYD88<sup>L265P</sup>.<sup>44</sup> Activated BTK coimmunoprecipitates with MYD88 that could be abrogated by use of a BTK kinase inhibitor, and overexpression of MYD88<sup>L265P</sup>, but not WT MYD88, triggers BTK activation. Knockdown of MYD88 by lentiviral transfection or use of a MYD88 homodimerization inhibitor also abrogated BTK activation in MYD88<sup>L265P</sup>-mutated WM cells. MYD88 also triggers HCK, a SRC family member that regulates AKT and ERK survival signaling, and also activates BTK itself.<sup>51</sup>

### CXCR4<sup>WHIM</sup> Mutations

The second most common somatic mutation after MYD88<sup>L265P</sup> revealed by WGS was found in the C-terminus of the CXCR4 receptor. These mutations are present in 30 to 35 percent of WM patients and impact serine phosphorylation sites that regulate CXCR4 signaling by its only known ligand, stromal cell-derived factor (SDF) 1a (CXCL12).<sup>29,52–54</sup>

The location of somatic mutations found in the C-terminus of CXCR4 in WM are similar to those observed in the germline of patients with WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome, a congenital immunodeficiency disorder characterized by chronic noncyclic neutropenia.<sup>55</sup> Patients with WHIM syndrome exhibit impaired CXCR4 receptor internalization following SDF-1a stimulation, which results in persistent CXCR4 activation and myelokathexis.<sup>56</sup>

In WM patients, two classes of CXCR4 mutations occur in the C-terminus. These include nonsense (CXCR4<sup>WHIM/NS</sup>) mutations, which truncate the distal 15- to 20-amino-acid region; and frameshift (CXCR4<sup>WHIM/FS</sup>) mutations, which compromise a region of up to 40 amino acids in the C-terminal domain.<sup>29,52</sup> Nonsense and frameshift mutations are almost equally divided among WM patients with CXCR4 somatic mutations, and more than 30 different types of CXCR4<sup>WHIM</sup> mutations have been identified in WM patients.<sup>29,52,54</sup> Preclinical studies with WM cells engineered to express nonsense and frameshift CXCR4<sup>WHIM</sup>-mutated receptors have shown enhanced and sustained AKT and extracellular signal-regulated kinase (ERK) signaling following SDF-1a relative to CXCR4<sup>WT</sup> (see Fig. 20-2), as well as increased cell migration, adhesion, growth and survival, and drug resistance of WM cells.<sup>53,57,58</sup>

### Other Somatic Events

Many copy number alterations have been revealed in WM patients; these alterations impact growth and survival pathways. Frequent loss of *HIVEP2* (80 percent) and *TNFAIP3* (50 percent) genes that are negative regulators of NF-κB expression (see Fig. 20-2), as well as *LYN* (70 percent) and *IBTK* (40 percent) that modulate B-cell receptor (BCR) signaling, have been revealed by WGS.<sup>29</sup> WGS has also revealed common defects in chromatin remodeling, with somatic mutations in *ARID1A* present in 17 percent and loss of *ARID1B* in 70 percent of WM patients. Both *ARID1A* and *ARID1B* are members of the SWI/SNF family of proteins and are thought to exert their effects via p53 and *CDKN1A* regulation. *TP53* is mutated in 7 percent of sequenced WM

genomes, while PRDM2 and TOP1 that participate in TP53-related signaling are deleted in 80 percent and 60 percent of WM patients, respectively.<sup>29</sup> Taken together, somatic events that contribute to impaired DNA damage response are also common in WM.

### Impact of Waldenström Macroglobulinemia Genomics on Clinical Presentation

MYD88 and CXCR4 mutations are important determinants of the clinical presentation of WM patients. Significantly higher marrow disease involvement, serum IgM levels, and symptomatic disease requiring therapy, including hyperviscosity syndrome, were observed in those patients with MYD88<sup>L265P</sup>CXCR4<sup>WHIM/NS</sup> mutations.<sup>52</sup> Patients with MYD88<sup>L265P</sup>CXCR4<sup>WHIM/FS</sup> or MYD88<sup>L265P</sup>CXCR4<sup>WT</sup> had intermediate marrow and serum IgM levels; those with MYD88<sup>WT</sup>CXCR4<sup>WT</sup> showed the lowest marrow disease burden. Fewer patients with MYD88<sup>L265P</sup> and CXCR4<sup>WHIM/FS</sup> or CXCR4<sup>WHIM/NS</sup>, compared to MYD88<sup>L265P</sup>CXCR4<sup>WT</sup>, presented with adenopathy, further delineating differences in disease tropism based on CXCR4 status. Despite the more aggressive presentation associated with CXCR4<sup>WHIM/NS</sup> genotype, risk of death was not impacted by CXCR4 mutation status. Risk of death was found to be 10-fold higher in patients with MYD88<sup>WT</sup> versus MYD88<sup>L265P</sup> genotype.<sup>52</sup>

## MARROW MICROENVIRONMENT

Increased numbers of mast cells are found in the marrow of WM patients, wherein they are usually admixed with tumor cell aggregates (see Fig. 20-1).<sup>14,18,59</sup> The role of mast cells in WM was investigated in one study, wherein coculture of primary autologous or mast cell lines with WM cells resulted in dose-dependent WM cell proliferation and/or tumor colony formation, through CD40 ligand (CD40L) signaling.<sup>59</sup> WM cells release soluble CD27 (sCD27), which may be triggered by cleavage of membrane-bound CD27 by matrix metalloproteinase 8 (MMP8).<sup>60</sup> sCD27 levels are elevated in the serum of WM patients and follow disease burden in mice engrafted with WM cells, as well as in WM patients.<sup>61</sup> sCD27 triggers the upregulation of CD40L and a proliferation-inducing ligand (APRIL) on mast cells derived from WM patients and mast cell lines through its receptor CD70. Modeling in mice engrafted with a CD70-blocking antibody shows inhibition of tumor cell growth, suggesting that WM cells require a microenvironmental support system for their growth and survival.<sup>61</sup> High levels of CXCR4 and very-late antigen-4 (VLA-4) have also been observed in WM cells.<sup>62</sup> In blocking experiment studies, CXCR4 supported migration of WM cells, while VLA-4 contributed to adhesion of WM cells to marrow stromal cells.<sup>62</sup>

## CLINICAL FEATURES

Table 20-1 presents the clinical and laboratory findings at time of diagnosis of WM in one large institutional study.<sup>16</sup> Unlike most indolent lymphomas, splenomegaly and lymphadenopathy are uncommon ( $\leq 15$  percent). Purpura is frequently associated with cryoglobulinemia and in rare circumstances with light-chain (AL) amyloidosis (Chap. 19). Hemorrhagic and neuropathic manifestations are multifactorial (see the “Immunoglobulin M-Related Neuropathy” section). The morbidity associated with WM is caused by the concurrence of two main components: tissue infiltration by neoplastic cells and, importantly, the physicochemical and immunologic properties of the monoclonal IgM. As shown in Table 20-2, the monoclonal IgM can produce clinical manifestations through several different mechanisms related to its physicochemical properties, nonspecific interactions with other proteins, antibody activity, and tendency to deposit in tissues.<sup>63-65</sup>

**TABLE 20-1.** Clinical and Laboratory Findings for 356 Consecutive Newly Diagnosed Patients with Waldenström Macroglobulinemia

|                                         | Median  | Range      | Normal Reference Range |
|-----------------------------------------|---------|------------|------------------------|
| Age (years)                             | 58      | 32–91      | NA                     |
| Gender (male/female)                    | 215/141 |            | NA                     |
| Marrow involvement (% of area on slide) | 30      | 5–95       | NA                     |
| Adenopathy (% of patients)              | 15      |            | NA                     |
| Splenomegaly (% of patients)            | 10      |            | NA                     |
| IgM (mg/dL)                             | 2620    | 270–12,400 | 40–230                 |
| IgG (mg/dL)                             | 674     | 80–2770    | 700–1600               |
| IgA (mg/dL)                             | 58      | 6–438      | 70–400                 |
| Serum viscosity (cp)                    | 2.0     | 1.1–7.2    | 1.4–1.9                |
| Hematocrit (%)                          | 35      | 17–45      | 35–44                  |
| Platelet count ( $\times 10^9/L$ )      | 275     | 42–675     | 155–410                |
| White cell count ( $\times 10^9/L$ )    | 6.4     | 1.7–22     | 3.8–9.2                |
| $\beta_2$ M (mg/dL)                     | 2.5     | 0.9–13.7   | 0–2.7                  |
| LDH (U/mL)                              | 313     | 61–1701    | 313–618                |

$\beta_2$ M,  $\beta_2$ -microglobulin; cp, centipoise; Ig, immunoglobulin; LDH, lactic dehydrogenase; NA, not applicable.

Data from patients seen at the Dana Farber Cancer Institute, Boston, MA.

## MORBIDITY MEDIATED BY THE EFFECTS OF IMMUNOGLOBULIN M

### Hyperviscosity Syndrome

The increased plasma IgM levels leads to blood hyperviscosity and its complications.<sup>66</sup> The mechanisms behind the marked increase in the resistance to blood flow and the resulting impaired transit through the microcirculatory system are complex.<sup>66-69</sup> The main determinants are (1) a high concentration of monoclonal IgMs, which may form aggregates and may bind water through their carbohydrate component; and (2) their interaction with blood cells. Monoclonal IgM increases red cell aggregation (rouleaux formation; Fig. 20-1) and red cell internal viscosity while reducing red cell deformability. The presence of cryoglobulins contributes to increasing blood viscosity, as well as to the tendency to induce erythrocyte aggregation. Serum viscosity is proportional to IgM concentration up to 30 g/L, then increases sharply at higher levels. Increased plasma viscosity may also contribute to inappropriately low erythropoietin production, which is the major reason for anemia in these patients.<sup>69</sup> Renal synthesis of erythropoietin is inversely correlated with plasma viscosity. Clinical manifestations are related to circulatory disturbances that can be best appreciated by ophthalmoscopy, which shows distended and tortuous retinal veins, hemorrhages, and papilledema (Fig. 20-3).<sup>70</sup> Symptoms usually occur

**TABLE 20–2.** Physicochemical and Immunological Properties of the Monoclonal Immunoglobulin M Protein in Waldenström Macroglobulinemia

| Properties of IgM Monoclonal Protein                                                                                                 | Diagnostic Condition       | Clinical Manifestations                                                                                                                                                       |
|--------------------------------------------------------------------------------------------------------------------------------------|----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Pentameric structure                                                                                                                 | Hyperviscosity             | Headaches, blurred vision, epistaxis, retinal hemorrhages, leg cramps, impaired mentation, intracranial hemorrhage                                                            |
| Precipitation on cooling                                                                                                             | Cryoglobulinemia (type I)  | Raynaud phenomenon, acrocyanosis, ulcers, purpura, cold urticaria                                                                                                             |
| Autoantibody activity to myelin-associated glycoprotein, ganglioside M <sub>1</sub> , sulfatide moieties on peripheral nerve sheaths | Peripheral neuropathies    | Sensorimotor neuropathies, painful neuropathies, ataxic gait, bilateral foot drop                                                                                             |
| Autoantibody activity to IgG                                                                                                         | Cryoglobulinemia (type II) | Purpura, arthralgia, renal failure, sensorimotor neuropathies                                                                                                                 |
| Autoantibody activity to red blood cell antigens                                                                                     | Cold agglutinins           | Hemolytic anemia, Raynaud phenomenon, acrocyanosis, livedo reticularis                                                                                                        |
| Tissue deposition as amorphous aggregates                                                                                            | Organ dysfunction          | Skin: bullous skin disease, papules, Schnitzler syndrome<br>Gastrointestinal: diarrhea, malabsorption, bleeding<br>Kidney: proteinuria, renal failure (light-chain component) |
| Tissue deposition as amyloid fibrils (light-chain component most commonly)                                                           | Organ dysfunction          | Fatigue, weight loss, edema, hepatomegaly, macroglossia, organ dysfunction of involved organs (heart, kidney, liver, peripheral sensory and autonomic nerves)                 |

Ig, immunoglobulin.

when the monoclonal IgM concentration exceeds 50 g/L or when serum viscosity is greater than 4.0 centipoises (cp), but there is individual variability, with some patients showing no evidence of hyperviscosity even at 10 cp.<sup>66</sup> The most common symptoms are oronasal mucosal bleeding, visual disturbances because of retinal bleeding, and dizziness that rarely may lead to stupor or coma. Heart failure can be aggravated, particularly in the elderly, owing to increased blood viscosity, expanded plasma volume, and anemia. Inappropriate red cell transfusion can exacerbate hyperviscosity and may precipitate cardiac failure.



**Figure 20–3.** Fundusoscopic examination of a patient with Waldenström macroglobulinemia with hyperviscosity-related changes, including dilated retinal vessels, hemorrhages, and “venous sausageing.” The white material at the edge of the veins may be cryoglobulin. (Used with permission of Marvin J. Stone, MD.)

### Cryoglobulinemia

The monoclonal IgM can behave as a cryoglobulin in up to 20 percent of patients and is usually type I and asymptomatic in most cases.<sup>16,66,71</sup> Cryoprecipitation is mainly dependent on the concentration of monoclonal IgM; for this reason, plasmapheresis or plasma exchange are commonly effective in this condition. Symptoms result from impaired blood flow in small vessels and include Raynaud phenomenon; acrocyanosis, and necrosis of the regions most exposed to cold, such as the tip of the nose, ears, fingers, and toes (Fig. 20–4); malleolar ulcers; purpura; and cold urticaria. Renal manifestations are infrequent. Mixed cryoglobulins (type II) consisting of IgM-IgG complexes may be associated with hepatitis C infections.<sup>71</sup>

### Autoantibody Activity

Monoclonal IgM may exert its pathogenic effects through specific recognition of autologous antigens, the most notable being nerve constituents, immunoglobulin determinants, and red blood cell antigens.

### Immunoglobulin M–Related Neuropathy

IgM-related peripheral neuropathy is common in WM patients, with estimated prevalence rates of 5 to 40 percent.<sup>72–74</sup> Approximately 8 percent of idiopathic neuropathies are associated with a monoclonal gammopathy, with a preponderance of IgM (60 percent), followed by IgG (30 percent) and IgA (10 percent) (Chap. 17).<sup>75,76</sup> The nerve damage is mediated by diverse pathogenetic mechanisms: (1) IgM antibody activity toward nerve constituents causing demyelinating polyneuropathies; (2) endoneurial granulo-fibrillar deposits of IgM without antibody activity, associated with axonal polyneuropathy; (3) occasionally by tubular deposits in the endoneurium associated with IgM cryoglobulin; and, rarely, (4) by amyloid deposits or by neoplastic cell infiltration of nerve structures.<sup>74,77</sup> Half of the patients with IgM neuropathy have a distinctive clinical syndrome that is associated with antibodies against a minor 100-kDa glycoprotein component of nerve known as the myelin-associated glycoprotein (MAG). Anti-MAG antibodies are generally monoclonal IgM $\kappa$  and usually also exhibit reactivity with other glycoproteins or glycolipids that share antigenic determinants with MAG.<sup>78–80</sup> The anti-MAG-related neuropathy





**Figure 20-4.** Cryoglobulinemia manifesting with severe acrocyanosis in a patient with Waldenström macroglobulinemia before (A) and following warming and plasmapheresis (B).

is typically distal and symmetrical, affecting both motor and sensory functions; it is slowly progressive with a long period of stability.<sup>73,81</sup> Most patients present with sensory complaints (paresthesias, aching discomfort, dysesthesias, or lancinating pains), imbalance and gait ataxia, owing to lack proprioception; leg muscles atrophy in advanced stage. Patients with predominantly demyelinating sensory neuropathy in association with monoclonal IgM to gangliosides with disialosyl moieties, such as GD1b, GD3, GD2, GT1b, and GQ1b, have also been reported.<sup>82,83</sup> Anti-GD1b and anti-GQ1b antibodies were associated with sensory ataxic neuropathy. These antiganglioside monoclonal IgMs present core clinical features of chronic ataxic neuropathy, sometimes with present ophthalmoplegia and/or red blood cell cold-agglutinating activity. The disialosyl epitope is also present on red blood cell glycoporphins, thereby accounting for the red cell cold agglutinin activity of anti-Pr2 specificity.<sup>84,85</sup> Monoclonal IgM proteins that bind to gangliosides with a terminal trisaccharide moiety, including ganglioside M<sub>2</sub> (GM<sub>2</sub>) and GalNac-GD1A, are associated with chronic demyelinating neuropathy and severe sensory ataxia, unresponsive to glucocorticoids.<sup>86</sup> Antiganglioside IgM proteins may also cross-react with lipopolysaccharides of *Campylobacter jejuni*, whose infection is known to precipitate the Miller-Fisher syndrome, a variant of the Guillain-Barré syndrome.<sup>87</sup> Thus, molecular mimicry may play a role in this condition. Antisulfatide monoclonal IgM proteins, associated with sensory-sensorimotor neuropathy, have been detected in 5 percent of patients with IgM monoclonal gammopathy and neuropathy.<sup>88</sup> Motor neuron disease has been reported in patients with WM and monoclonal IgM with anti-GM<sub>1</sub> and sulfoglucuronyl paragloboside activity.<sup>89</sup> Polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes (the POEMS syndrome) are rare in patients with WM.<sup>90</sup>

### Cold Agglutinin Hemolytic Anemia

Monoclonal IgM may have cold agglutinin activity; that is, it can recognize specific red cell antigens at temperatures below 37°C, producing chronic hemolytic anemia. This disorder occurs in less than 10 percent of WM patients and is associated with cold agglutinin titers greater than

1:1000 in most cases.<sup>91</sup> The monoclonal component is usually an IgM $\kappa$  and reacts most commonly with red cell I/i antigens, resulting in complement fixation and activation.<sup>92</sup> Mild-to-moderate chronic hemolytic anemia can be exacerbated after cold exposure. Hemoglobin usually remains above 70 g/L. The hemolysis is usually extravascular, mediated by removal of C3b-opsonized red cells by the mononuclear phagocyte system, primarily in the liver. Intravascular hemolysis from complement destruction of red blood cell membrane is infrequent. The agglutination of red cells in the skin circulation also causes Raynaud syndrome, acrocyanosis, and livedo reticularis. Macroglobulins with the properties of both cryoglobulins and cold agglutinins with anti-Pr specificity can occur. These properties may have as a common basis the binding of the sialic acid-containing carbohydrate present on red blood cell glycoporphins and on Ig molecules. Several other macroglobulins with antibody activity toward autologous antigens (e.g., phospholipids, tissue and plasma proteins), and foreign ligands have also been described.

### Immunoglobulin M Tissue Deposition

The monoclonal protein can deposit in several tissues as amorphous aggregates. Linear deposition of monoclonal IgM along the skin basement membrane is associated with bullous skin disease.<sup>93</sup> Amorphous IgM deposits in the dermis result in IgM storage papules on the extensor surface of the extremities, referred to as macroglobulinemia cutis.<sup>94</sup> Deposition of monoclonal IgM in the lamina propria and/or submucosa of the intestine may be associated with diarrhea, malabsorption, and gastrointestinal bleeding.<sup>95,96</sup> Kidney involvement is less common and less severe in WM than in myeloma, probably because the amount of light chain excreted in the urine is generally lower in WM than in myeloma and because of the absence of contributing factors, such as hypercalcemia. Urinary cast nephropathy, however, has occurred in WM.<sup>97</sup> On the other hand, the IgM macromolecule is more susceptible to being trapped in the glomerular loops, where ultrafiltration presumably contributes to its precipitation, forming subendothelial deposits of aggregated IgM proteins that occlude the glomerular capillaries.<sup>98</sup> Mild and reversible

proteinuria may result, and most patients are asymptomatic. The deposition of monoclonal light chain as fibrillar amyloid deposits (AL amyloidosis) is uncommon in patients with WM.<sup>99</sup> Clinical expression and prognosis are similar to those of other AL amyloidosis patients with involvement of heart (44 percent), kidneys (32 percent), liver (14 percent), lungs (10 percent), peripheral or autonomic nerves (38 percent), and soft tissues (18 percent). The incidence of cardiac and pulmonary involvement is higher in patients with monoclonal IgM than with other immunoglobulin isotypes. The association of WM with reactive amyloidosis has been documented rarely.<sup>100,101</sup> Simultaneous occurrence of fibrillary glomerulopathy, characterized by glomerular deposits of wide nonconophilic fibrils and amyloid deposits, has been described.<sup>102</sup>

## MANIFESTATIONS RELATED TO TISSUE INFILTRATION BY NEOPLASTIC CELLS

Tissue infiltration by neoplastic cells is uncommon but can involve various organs and tissues, including the liver, spleen, lymph nodes, lungs, gastrointestinal tract, kidneys, skin, eyes, and central nervous system.

### Lung

Pulmonary involvement in the form of masses, nodules, diffuse infiltrate, or pleural effusions is uncommon; the overall incidence of pulmonary and pleural findings is approximately 4 percent.<sup>103–105</sup> Cough is the most common presenting symptom, followed by dyspnea and chest pain. Chest radiographic findings include parenchymal infiltrates, confluent masses, and effusions.

### Gastrointestinal Tract

Malabsorption, diarrhea, bleeding, or obstruction may indicate involvement of the gastrointestinal tract at the level of the stomach, duodenum, or small intestine.<sup>106–109</sup>

### Renal System

In contrast to myeloma, infiltration of the kidney interstitium with lymphoplasmacytoid cells can occur in WM, and renal or perirenal masses are not uncommon.<sup>110,111</sup>

### Skin

The skin can be the site of dense lymphoplasmacytoid infiltrates, similar to that seen in the liver, spleen, and lymph nodes, forming cutaneous plaques and, rarely, nodules.<sup>112</sup> Chronic urticaria and IgM gammopathy are the two cardinal features of the Schnitzler syndrome, which is not usually associated initially with clinical features of WM, although evolution to WM is not uncommon.<sup>113</sup> Thus, close followup of these patients is important.

### Joints

Invasion of articular and periarticular joint structures by WM malignant cells is rarely reported.<sup>114</sup>

### Eye

The neoplastic cells can infiltrate the periorbital structures, lacrimal gland, and retroorbital lymphoid tissues of the eye, resulting in ocular nerve palsies.<sup>115,116</sup>

### Central Nervous System

Direct infiltration of the central nervous system by monoclonal lymphoplasmacytoid cells as infiltrates or as tumors constitutes the rarely observed Bing-Neel syndrome, characterized clinically by confusion, memory loss, disorientation, and motor dysfunction (reviewed in Ref. 117).

## LABORATORY FINDINGS

### BLOOD ABNORMALITIES

Anemia is the most common finding in patients with symptomatic WM and is caused by a combination of factors: decrease in red cell survival, impaired erythropoiesis, moderate plasma volume expansion, hepcidin production leading to iron reutilization defect, and blood loss from the gastrointestinal tract.<sup>16,118,119</sup> Blood films are usually normocytic and normochromic, and rouleaux formation is often pronounced (see Fig. 20–1). Mean red cell volume may be elevated spuriously owing to erythrocyte aggregation. Leukocyte and platelet counts are usually within the reference range at presentation, although patients may occasionally present with severe thrombocytopenia. Monoclonal B lymphocytes expressing surface IgM and late-differentiation B-cell markers are uncommonly detected in blood by flow cytometry. A raised erythrocyte sedimentation rate is almost always present and may be the first clue to the presence of the macroglobulinemia. The clotting abnormality detected most frequently is prolongation of thrombin time. AL amyloidosis should be suspected in all patients with nephrotic syndrome, cardiomyopathy, hepatomegaly, or peripheral neuropathy. Diagnosis requires the demonstration of green birefringence under polarized light of amyloid deposits stained with Congo red.

### MARROW FINDINGS

Central to the diagnosis of WM is the demonstration, by trephine biopsy, of marrow infiltration by a lymphoplasmacytoid cell population characterized by small lymphocytes with evidence of plasmacytoid and plasma cell maturation (see Fig. 20–1).<sup>1,14</sup> The pattern of marrow infiltration may be diffuse, interstitial, or nodular, usually with an intertrabecular pattern of infiltration. A solely paratrabecular pattern of infiltration is unusual and should raise the possibility of follicular lymphoma.<sup>1</sup> The marrow cell immunophenotype should be confirmed by flow cytometry and/or immunohistochemistry. The cell immunoprofile: sIgM<sup>+</sup>CD19<sup>+</sup>CD20<sup>+</sup>CD22<sup>+</sup>CD79<sup>+</sup> is characteristic of WM.<sup>14,120,121</sup> Up to 20 percent of cases may express CD5, CD10, or CD23.<sup>19</sup> In these cases, chronic lymphocytic leukemia and mantle cell lymphoma should be excluded. “Intranuclear” periodic acid Schiff–positive inclusions (Dutcher–Fahey bodies)<sup>122</sup> consisting of IgM deposits in the perinuclear space, and sometimes in intranuclear vacuoles, may be seen occasionally in lymphoid cells. An increased number of mast cells, usually in association with the lymphoid aggregates, is commonly found, and their presence may help in differentiating WM from other B-cell lymphomas (Fig. 20–5).<sup>14</sup> MYD88 mutation testing of marrow samples has been incorporated into many clinical laboratories and may help in clarifying the diagnosis of WM from other IgM-secreting entities.<sup>35–39</sup> The use of blood B cells may also permit determination of MYD88 mutation status by allele-specific PCR assays, particularly in untreated WM patients. Non-L265P MYD88 mutations should be excluded if a patient shows WT status by allele-specific PCR MYD88<sup>L265P</sup> assays.<sup>42</sup>

### IMMUNOLOGIC ABNORMALITIES

High-resolution electrophoresis combined with immunofixation of serum and urine is recommended for identification and characterization of the IgM monoclonal protein. The light chain of the monoclonal IgM is  $\kappa$  in 75 to 80 percent of patients. More than one M component may be present. The concentration of the serum monoclonal protein is very variable but in most cases lies within the range of 15 to 45 g/L. Densitometry should be adopted to determine IgM levels for serial evaluations because nephelometry is unreliable and shows large laboratory variation. The presence of cold agglutinins or cryoglobulins may affect

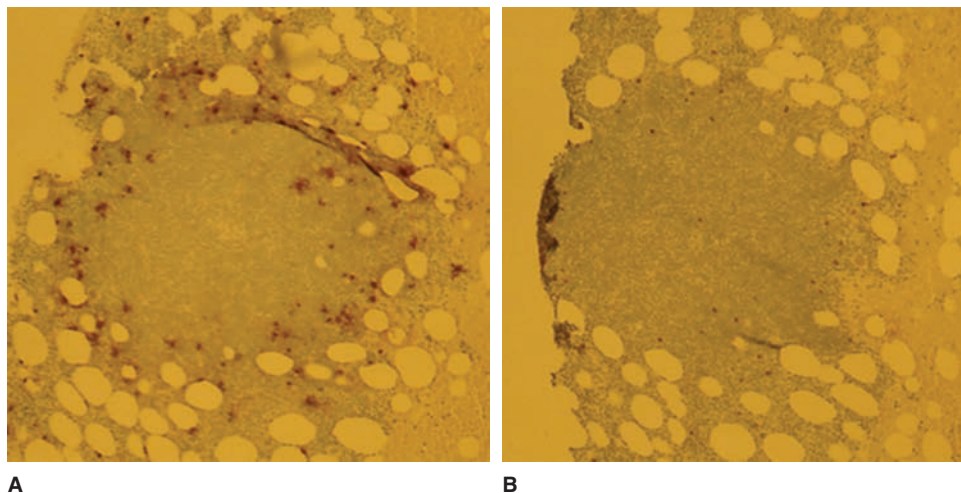


**Figure 20-5.** Marrow clot section. **A.** Tryptase-staining mast cells surrounding a nodule of lymphoplasmacytic cells in a patient with Waldenström macroglobulinemia. **B.** Mast cells in the same section exhibit strong CD40 ligand signaling, which has been shown to support (at least in part) the growth and survival of lymphoplasmacytic cells.

determination of IgM levels; therefore, testing for cold agglutinins and cryoglobulins should be performed at diagnosis. If present, subsequent serum samples should be analyzed at 37°C for determination of serum monoclonal IgM level. Although Bence Jones proteinuria is frequently present, it exceeds 1 g/24 hours in only 3 percent of cases. Whereas IgM levels are elevated in WM patients, IgA and IgG levels are most often depressed and do not recover after successful treatment.<sup>123</sup>

## SERUM VISCOSITY

Because of its large size (almost 1 million daltons), most IgM molecules are retained within the intravascular compartment and can exert an undue effect on serum viscosity.<sup>64</sup> Serum viscosity can be measured if the patient has signs or symptoms of hyperviscosity syndrome, although serum viscosity levels are erratic because of a lack of standardization in most clinical laboratories.<sup>16</sup> As such, inferences derived from serum IgM levels may be more reliable. Patients typically become symptomatic at serum viscosity levels of 4 cp and above, which relates to serum IgM levels above 6000 mg/dL.<sup>124,125</sup> Patients may be symptomatic at lower serum viscosity and IgM levels, and in these patients cryoglobulins may be present. Recurring nosebleeds, headaches, and visual disturbances are common symptoms in patients with symptomatic hyperviscosity.<sup>16</sup> Funduscopy is an important indicator of clinically relevant hyperviscosity. Among the first clinical signs of hyperviscosity are the appearance of peripheral and midperipheral dot and blot-like hemorrhages in the retina, which are best appreciated with indirect ophthalmoscopy and scleral depression.<sup>70</sup> In more severe cases of hyperviscosity, dot, blot, and flame-shaped hemorrhages can appear in the macular area along with markedly dilated and tortuous veins with focal constrictions, resulting in “venous saussaging,” as well as papilledema. The effects of hyperviscosity are mediated by the blood viscosity as a result of IgM–red cell interactions, but measurement of serum viscosity is much simpler than is blood viscosity and is not shear rate dependent so it is easier to related to clinical manifestations.

## IMAGING

Magnetic resonance imaging (MRI) of the spine in conjunction with computed tomography (CT) of the abdomen and pelvis are useful in evaluating disease status.<sup>126</sup> Marrow involvement can be documented

by MRI studies of the spine in more than 90 percent of patients; CT of the abdomen and pelvis demonstrates enlarged nodes in approximately 40 percent of WM patients.<sup>126</sup>

## LYMPH NODE BIOPSY

Lymph node biopsy may show preserved architecture or replacement by infiltration of neoplastic cells with lymphoplasmacytoid, lymphoplasmacytic, or polymorphous cytologic patterns.

## TREATMENT

### DECIDING ON INITIATING TREATMENT

As part of the Second International Workshop on Waldenström Macroglobulinemia, a consensus panel was organized to recommend criteria for the initiation of therapy in patients with WM.<sup>127</sup> The panel recommended that initiation of therapy should not be based on the IgM level per se, as this may not correlate with the clinical manifestations of WM. The consensus panel did, however, agree that initiation of therapy is appropriate for patients with constitutional symptoms, such as recurrent fever, night sweats, fatigue as a consequence of anemia, or weight loss. Progressive symptomatic lymphadenopathy and/or splenomegaly provide additional reasons to begin therapy. Anemia with a hemoglobin value of 10 g/dL or less or a platelet count of  $100 \times 10^9/L$  or less owing to marrow infiltration also justifies treatment. Certain complications, such as hyperviscosity syndrome, symptomatic sensorimotor peripheral neuropathy, systemic amyloidosis, renal insufficiency, or symptomatic cryoglobulinemia, may also be indications for therapy.<sup>16,127</sup>

## INITIAL THERAPY

The International Workshops on Waldenström Macroglobulinemia have also formulated consensus recommendations for both initial therapy and therapy for refractory disease based on the best available evidence. The recommendations emerged from the Eighth International Workshop on Waldenström’s Macroglobulinemia.<sup>128</sup> Individual patient considerations, including the presence of cytopenias, need for more rapid disease control, age, and candidacy for autologous transplant therapy, should be taken into account in making the choice of the



drugs to use. For patients who are candidates for autologous stem cell transplantation (SCT), which typically is reserved for those patients younger than 70 years of age, the panel recommended that exposure to alkylating agents or nucleoside analogues should be limited. The use of nucleoside analogues should be approached cautiously in patients with WM as there appears to be an increased risk for the development of disease transformation, as well as myelodysplasia and acute myelogenous leukemia.

### Oral Alkylating Agents

Oral alkylating drugs, alone and in combination therapy with glucocorticoids, have been extensively evaluated in the treatment of WM. Chlorambucil has been administered on both a continuous (i.e., daily dose schedule) and an intermittent schedule. Patients receiving chlorambucil on a continuous schedule typically receive 0.1 mg/kg per day, whereas on the intermittent schedule patients typically receive 0.3 mg/kg for 7 days, every 6 weeks. In a prospective randomized study, no significant difference in the overall response rate between these schedules was observed,<sup>129</sup> although the median response duration was greater for patients receiving intermittent-dose versus continuous-dose chlorambucil (46 vs. 26 months). Despite the favorable median response duration in this study for use of the intermittent schedule, no difference in the median overall survival was observed. Moreover, an increased incidence for development of myelodysplasia and acute myelogenous leukemia with the intermittent (three of 22 patients) versus the continuous (zero of 24 patients) chlorambucil schedule prompted the preference for use of continuous chlorambucil dosing. The use of glucocorticoids in combination with alkylating agent therapy has also been explored. Chlorambucil (8 mg/m<sup>2</sup>) plus prednisone (40 mg/m<sup>2</sup>) given orally for 10 days, every 6 weeks, resulted in a major response (i.e., reduction of IgM by more than 50 percent) in 72 percent of patients.<sup>130</sup> Alkylating agent regimens employing melphalan and cyclophosphamide in combination with glucocorticoids also have been examined.<sup>131,132</sup> This approach produced slightly higher overall response rates and response durations, although the benefit of these more complex regimens over chlorambucil remains to be demonstrated. Pretreatment factors associated with shorter survival in the entire population of patients receiving single-agent chlorambucil were age older than age 60 years, male sex, hemoglobin less than 10 g/dL, leukocytes less than  $4 \times 10^9/L$ , and platelets less than  $150 \times 10^9/L$ . Organomegaly, signs of hyperviscosity, renal failure, monoclonal IgM level, blood lymphocytosis, and percentage of marrow lymphoid cells were not significantly correlated with survival.<sup>133</sup> Additional factors to be taken into account in considering alkylating agent therapy for patients with WM include necessity for more rapid disease control given the slow response, as well as consideration for preserving stem cells in patients who are candidates for autologous SCT therapy. A large randomized study showed an inferior response rate and time to progression in WM patients receiving chlorambucil versus fludarabine, as well as a higher incidence of secondary malignancies in the former. Neutropenia was, however, more pronounced in those patients on fludarabine.<sup>134</sup>

### Nucleoside Analogue Therapy

Cladribine administered as a single agent by continuous intravenous infusion, by 2-hour daily infusion, or by subcutaneous bolus injections for 5 to 7 days has resulted in major responses in 40 to 90 percent of patients who received primary therapy, whereas in the previously treated patients, responses have ranged from 38 to 54 percent.<sup>135–141</sup> Median time to achievement of response in responding patients following cladribine ranged from 1.2 to 5 months. The overall response rate with daily infusion of fludarabine, administered mainly on 5-day schedules, in previously untreated and treated patients ranged from 38 to 100 percent

and 30 to 40 percent, respectively,<sup>142–147</sup> similar to the responses to cladribine. Median time to achievement of response for fludarabine (3 to 6 months) was also similar to cladribine. In general, response rates and durations of responses have been greater for patients receiving nucleoside analogues as initial therapy, although in several studies in which both untreated and previously treated patients were enrolled, no difference in the overall response rate was reported.

Myelosuppression commonly occurs following prolonged exposure to either of the nucleoside analogues. A sustained decrease in both CD4+ and CD8+ T lymphocytes, measured 1 year following initiation of therapy, is notable.<sup>135–137</sup> Treatment-related mortality as a consequence of myelosuppression and/or opportunistic infections attributable to immunosuppression occurred in up to 5 percent of all treated patients in some series with nucleoside analogues.

Factors predicting for a better response to nucleoside analogues include younger age at start of treatment (<70 years), higher pretreatment hemoglobin (>95 g/L), higher platelet count (> $75 \times 10^9/L$ ), disease relapsing off therapy, and a long interval between first-line therapy and initiation of a nucleoside analogue in relapsing patients.<sup>135,140,146</sup> There are limited data on the use of an alternate nucleoside analogue in previously treated patients among whom disease relapsed or who had resistance when not on cladribine or fludarabine therapy.<sup>148,149</sup> Three of four (75 percent) patients responded to cladribine after progression following an unmaintained remission to fludarabine, whereas only one of 10 (10 percent) with disease resistant to fludarabine responded to cladribine.<sup>148</sup> A response in two of six patients (33 percent) and disease stabilization in the remaining patients to fludarabine, in spite of an inadequate response or progressive disease, following cladribine therapy has been reported.<sup>149</sup>

Harvesting autologous blood stem cells succeeded on the first attempt in 14 of 15 patients who did not receive nucleoside analogue therapy as compared to two of six patients who received a nucleoside analogue.<sup>150</sup> A sevenfold increase in transformation to an aggressive lymphoma and a threefold increase in the development of myelodysplasia or acute myelogenous leukemia were observed among patients who received a nucleoside analogue versus other therapies for their WM.<sup>151</sup> A meta-analysis of several trials in which patients were treated with nucleoside analogues in WM patients included patients who had previously received an alkylating agent and showed a crude incidence of approximately 8 percent for development of disease transformation and of approximately 5 percent for development of myelodysplasia or acute myelogenous leukemia.<sup>152</sup> None of the risk factors—that is, gender, age, family history of WM, or B-cell malignancies, typical markers of tumor burden and prognosis, type of nucleoside analogue therapy (cladribine vs. fludarabine), time from diagnosis to nucleoside analogue use, nucleoside analogue treatment as primary or salvage therapy, or treatment with an oral alkylator (i.e., chlorambucil)—predicted for the occurrence of transformation or development of myelodysplasia or acute myelogenous leukemia in patients treated with a nucleoside analogue.<sup>152</sup>

### CD20-Directed Antibody Therapy

Rituximab is a chimeric monoclonal antibody that targets CD20, a widely expressed antigen on lymphoplasmacytic cells in WM.<sup>153</sup> Several retrospective and prospective studies indicated that rituximab, when used at standard doses (i.e., 4 weekly intravenous infusions of 375 mg/m<sup>2</sup>) induced major responses in approximately 30 percent of previously treated and untreated patients.<sup>154,155</sup> Even patients who achieved minor responses benefited from rituximab by improved hemoglobin and platelet counts and reduction of lymphadenopathy and/or splenomegaly.<sup>154</sup> The median time to treatment failure in these studies was found to range from 8 to 27+ months. Patients on an extended rituximab schedule consisting of four weekly courses at 375 mg/m<sup>2</sup> per week, repeated

3 months later by another 4-week course, have demonstrated major response rates of approximately 45 percent, with time-to-progression estimates of 16+ to 29+ months.<sup>156,157</sup>

In many WM patients, a transient increase or flare of the serum IgM may occur immediately following initiation of rituximab treatment.<sup>156,158,159</sup> Such an increase does not herald treatment failure, and most patients will return to their baseline serum IgM level by 12 weeks. Some patients continue to show a prolonged increase in IgM despite an apparent reduction in their marrow tumor cells. However, patients with baseline serum IgM levels of greater than 50 g/dL or serum viscosity of greater than 3.5 cp may be particularly at risk for a hyperviscosity-related event, and plasmapheresis should be considered in these patients in advance of rituximab therapy.<sup>158</sup> Because of the decreased likelihood of response in patients with higher IgM levels, as well as the possibility that serum IgM and blood viscosity levels may abruptly rise, rituximab monotherapy should not be used as sole therapy for the treatment of patients at risk for hyperviscosity signs and symptoms.<sup>128,156,157</sup>

Time to response after rituximab is slow and exceeds 3 months on the average. The time to best response in one study was 18 months.<sup>157</sup> Patients with baseline serum IgM levels of less than 60 g/dL are more likely to respond, regardless of the underlying marrow involvement by tumor cells.<sup>156,157</sup> An analysis of 52 patients who were treated with single-agent rituximab found the objective response rate was significantly lower in patients who had either low serum albumin (<35 g/L) or a serum monoclonal protein greater than 40 g/L.<sup>160</sup> The presence of both adverse prognostic factors was associated with a short time to progression (3.6 months). Patients who had normal serum albumin and relatively low serum monoclonal protein levels derived a substantial benefit from rituximab with a time to progression exceeding 40 months.

A correlation between polymorphisms at position 158 in the FcγRIIIa receptor (CD16), an activating Fc receptor on important effector cells that mediate antibody-dependent cell-mediated cytotoxicity, and rituximab response was observed in WM patients.<sup>161</sup> Individuals may encode either the amino acid valine or phenylalanine at position 158 in the FcγRIIIa receptor. WM patients who carried the valine amino acid (in either a homozygous or a heterozygous pattern) had a fourfold higher major response rate (i.e., 50 percent decline in serum IgM levels) to rituximab versus those patients who expressed phenylalanine in a homozygous pattern.

### Proteasome Inhibitors

Both bortezomib and carfilzomib have been evaluated in prospective studies in patients with WM, although the latter only in combination therapy (discussed in "Combination Therapies"). In a retrospective study, 10 patients with refractory or relapsed WM were treated with bortezomib administered intravenously at a dose of 1.3 mg/m<sup>2</sup> on days 1, 4, 8, and 11 in a 21-day cycle for a total of four cycles. Most patients had been exposed to all active agents for WM, and eight patients had received three or more regimens. Six of these patients achieved a partial response, which occurred at a median of 1 month. The median time to progression in the responding patients is expected to exceed 11 months. Peripheral neuropathy occurred in three patients, and one patient developed severe paralytic ileus in this series.<sup>162</sup> In a prospective study among 27 relapsed or refractory patients who received up to eight cycles of bortezomib at 1.3 mg/m<sup>2</sup> on days 1, 4, 8, and 11, median serum IgM levels declined significantly from 4.7 g/dL to 2.1 g/dL.<sup>33</sup> The overall response rate was 85 percent, with 10 and 13 patients achieving a minor (≥25 percent) and major (≥50 percent) decrease in IgM level. Responses occurred at a median of 1.4 months. The median time to progression for all responding patients in this study was 7.9 (range: 3.0 to 21.4+) months, and the most common grades III/IV toxicities were sensory neuropathies (22.2 percent), leukopenia (18.5 percent), neutropenia (14.8 percent),

dizziness (11.1 percent), and thrombocytopenia (7.4 percent). Sensory neuropathies resolved or improved in nearly all patients following cessation of therapy. Twenty-seven patients with both untreated (44 percent) and previously treated (56 percent) disease received bortezomib, using the standard schedule until they either demonstrated progressive disease or were two cycles beyond a complete response or stable disease.<sup>163</sup> The overall response rate was 78 percent, with major responses observed in 44 percent of patients. Sensory neuropathy occurred in 20 patients following two to four cycles of therapy. Among the 20 patients developing a neuropathy, 14 showed resolution or improvement 2 to 13 months after therapy.

### Combination Therapies

Because rituximab is not myelosuppressive, its combination with chemotherapy has been explored. A regimen of rituximab, cladribine, and cyclophosphamide used in 17 previously untreated patients resulted in a partial response in 94 percent of WM patients, including a complete response in 18 percent.<sup>164</sup> No patient had relapsed with a median followup of 21 months. The combination of rituximab and fludarabine used in 43 patients, of whom 32 (75 percent) were previously untreated, led to an overall response rate of 95.3 percent, with 83 percent of patients achieving a major response (i.e., 50 percent reduction in disease burden).<sup>165</sup> The median time to progression was 51.2 months in this series and was longer for those patients who were previously untreated and for those achieving a very good partial remission (i.e., 90 percent reduction in disease) or better. Hematologic toxicity was common: Grade 3 neutropenia and thrombocytopenia were observed in 27 and four patients, respectively. Two deaths occurred from pneumonia in this study. Secondary malignancies, including transformation to aggressive lymphoma and development of myelodysplasia or acute myelogenous leukemia, were observed in six patients in this series. The addition of rituximab to fludarabine and cyclophosphamide has also been explored in previously treated patients, of whom four of five patients had a response.<sup>166</sup> In another combination study, rituximab along with pentostatin and cyclophosphamide given to 13 patients with untreated and previously treated WM or LPL resulted in a major response in 77 percent of patients.<sup>167</sup> The combination of rituximab, dexamethasone, and cyclophosphamide was used as primary therapy to treat 72 patients with WM, in whom a major response was observed in 74 percent of patients in this study, and the 2-year progression-free survival was 67 percent.<sup>168</sup> Therapy was well tolerated, although one patient died of interstitial pneumonia.

Two studies examined cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in combination with rituximab (R-CHOP). In a randomized trial involving 69 patients, most of whom had WM, the addition of rituximab to CHOP resulted in a higher overall response rate (94 percent vs. 67 percent) and median time to progression (63 vs. 22 months) in comparison to patients treated with CHOP alone.<sup>169</sup> R-CHOP was also used in 13 WM patients, 10 of whom had relapsed or refractory disease.<sup>170</sup> Among 13 evaluable patients, 10 patients achieved a major response (77 percent), including three complete and seven partial remissions. Two other patients achieved a minor response. In a retrospective study of symptomatic WM patients who received R-CHOP; rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP); or cyclophosphamide, prednisone, and rituximab (R-CP) and were similar in most pretreatment variables, the overall response rates to therapy were comparable among all three treatment groups—R-CHOP 96 percent, R-CVP 88 percent, and R-CP 95 percent—although there was a trend for more complete remissions among patients treated with R-CVP and R-CHOP.<sup>171</sup> Adverse events attributed to therapy showed a higher incidence for neutropenic fever and treatment-related neuropathy for R-CHOP and R-CVP versus R-CP. The results of this study suggest that,

in WM, the use of R-CP may provide analogous treatment responses to more intense cyclophosphamide-based regimens while minimizing treatment-related complications. The extended alkylator bendamustine has also been evaluated in combination with rituximab in both untreated and previously treated WM patients. A randomized study by the German STiL Group examined bendamustine plus rituximab (BR) versus R-CHOP in patients with untreated, indolent B-cell lymphomas, including WM.<sup>172</sup> Patients with WM in this study showed similar overall responses (96 percent vs. 94 percent), although progression-free survival was significantly longer (69 vs. 29 months) in patients who received BR versus R-CHOP. Treatment was also better tolerated in patients receiving BR. In the relapsed or refractory setting, an overall response rate of 83 percent was observed with bendamustine in combination with a CD20 monoclonal antibody.<sup>173</sup> The median time to progression was 13 months in this study. Prolonged myelosuppression was more common in patients who received prior nucleoside analogues.

The use of two cycles of oral cyclophosphamide along with subcutaneous cladribine to 37 patients with previously untreated WM led to a partial response in 84 percent of patients, and the median duration of response was 36 months.<sup>164</sup> Fludarabine in combination with intravenous cyclophosphamide resulted in partial responses in six of 11 (55 percent) WM patients with either primary refractory disease or who had relapsed on treatment.<sup>174</sup> The combination of fludarabine plus cyclophosphamide was also evaluated in 49 patients, 35 of whom were previously treated. Seventy-eight percent of the patients achieved a response, and the median time to treatment failure was 27 months.<sup>175</sup> Hematologic toxicity was frequent, and three patients died of treatment-related toxicities. Two important findings in this study were the development of acute leukemia in two patients, histologic transformation to diffuse large B-cell lymphoma in one patient, and two cases of solid malignancies (prostate and melanoma), as well as failure to mobilize stem cells in four of six patients.

The combination of bortezomib, dexamethasone, and rituximab (BDR) as primary therapy in patients with WM resulted in an overall response rate of 96 percent and a major response rate of 83 percent.<sup>176</sup> The incidence of grade 3 neuropathy was approximately 30 percent but was reversible in most patients following discontinuation of therapy. An increased incidence of herpes zoster was also observed, prompting the prophylactic use of antiviral therapy. The median progression-free survival in this study was 66 months.<sup>177</sup> Alternative schedules for administration of bortezomib (i.e., once weekly at higher doses) in combination with rituximab in patients with WM have achieved overall response rates of 80 to 90 percent.<sup>178,179</sup> The European Myeloma Network (EMN) recently showed that transitioning bortezomib from twice weekly intravenous dosing during the first cycle to weekly administration thereafter reduced grade 3 neuropathy to less than 10 percent in patients treated with BDR. The median progression-free survival in this study was 43 months.<sup>180</sup> There have been no studies addressing the safety and efficacy of subcutaneous bortezomib use in WM.

Carfilzomib, which is associated with a low risk of treatment-related peripheral neuropathy, has been evaluated in combination with rituximab and dexamethasone (CaRD) in WM patients.<sup>34</sup> Carfilzomib was administered intravenously at 20 mg/m<sup>2</sup> (cycle 1), then 36 mg/m<sup>2</sup> (cycles 2 to 6), together with dexamethasone (20 mg) on days 1, 2, 8, and 9 as part of a 21-day cycle. As part of this regimen, rituximab 375 mg/m<sup>2</sup> was given on days 2 and 9 every 21 days. Maintenance therapy was given 8 weeks following induction therapy with intravenous carfilzomib (36 mg/m<sup>2</sup>) and dexamethasone (20 mg) administered on days 1 and 2 and rituximab 375 mg/m<sup>2</sup> on day 2 every 8 weeks for up to eight cycles. Overall response rate with this regimen was 87.1 percent (one complete response, 10 very good partial responses, 10 partial responses, and six minimal responses) and was not impacted by

MYD88<sup>L265P</sup> or CXCR4<sup>WHIM</sup> mutation status. With a median followup of 15.4 months, 20 patients remained progression free. Grade 2 or higher toxicities included asymptomatic hyperlipasemia (41.9 percent), reversible neutropenia (12.9 percent), and cardiomyopathy in one patient (3.2 percent) with multiple risk factors. Treatment-related neuropathy, which was grade 2, occurred in one patient (3.2 percent). Declines in serum IgA and IgG were common, and some patients required intravenous  $\gamma$ -globulin therapy for recurring sinus and bronchial infections.

### Novel Therapeutics

The use of ibrutinib was recently approved by the FDA for the treatment of symptomatic patients with WM. Ibrutinib inhibits BTK and HCK, both of which are transactivated by mutated MYD88.<sup>44,51</sup> In a multicenter study that examined the role of ibrutinib in previously treated (median: two prior therapies, 40 percent refractory) WM patients, the overall response rate was 91 percent.<sup>181</sup> Patients in this study received 420 mg/day of ibrutinib by mouth. Posttherapy, median serum IgM levels declined from 3610 to 915 mg/dL; hemoglobin rose from 10.5 to 13.5 g/dL; and marrow involvement declined from 60 percent to 25 percent. Decreased or resolved adenopathy was observed in 60 percent of patients with extramedullary disease, and five of nine patients with IgM-related peripheral neuropathy had symptomatic improvement. The 24-month estimate for progression-free and overall survival was 70 percent and 95 percent, respectively. Although major response rates were lower in patients with MYD88<sup>WT</sup> and CXCR4<sup>WHIM</sup> mutations, it is not recommended that genotyping be used to select which patients should go on ibrutinib until further data are available. Grade 2 or higher treatment-related toxicities included neutropenia (25 percent) and thrombocytopenia (14 percent), which were more common in heavily pretreated patients; atrial fibrillation associated with a prior history of arrhythmia (5 percent); and bleeding associated with procedures and marine oil supplements (3 percent). Serum IgA and IgG levels were unchanged following treatment with ibrutinib, and treatment-related infections were infrequent. An overall response rate of 90% with major responses in 70% of patients was also observed in a study of ibrutinib in rituximab-refractory WM patients. The presence of CXCR4 mutations was also associated with slower IgM and hemoglobin response in this study.<sup>182</sup>

Everolimus is an oral inhibitor of the mammalian target of rapamycin (mTOR) pathway that is active in WM. A multicenter study examined everolimus in 60 previously treated patients that showed an overall response rate of 73 percent, with 50 percent of patients attaining a major response.<sup>182</sup> The median progression-free survival in this study was 21 months. Grade 3 or higher related toxicities were observed in 67 percent of patients, with cytopenias constituting the most common toxicity. Pulmonary toxicity occurred in 5 percent of patients, and dose reductions as a result of toxicity occurred in 52 percent of patients. A clinical trial examining the activity of everolimus in 33 previously untreated patients with WM has also been reported that included serial marrow biopsies in response assessment.<sup>183</sup> The ORR in this study was 72 percent, including partial or better responses in 60 percent of patients. However, discordance between serum IgM levels, upon which consensus criteria for response are based, and marrow disease response were common and complicated response assessment. In a few patients, discontinuation of everolimus led to rapid increases in serum IgM levels and symptomatic hyperviscosity. Grade 2 or higher hematologic and nonhematologic toxicities in this study that were related to everolimus were predominantly hematologic, including anemia (40 percent), thrombocytopenia (12 percent), and neutropenia (18 percent). Non-hematologic toxicities included oral ulcerations (27 percent), which improved with oral dexamethasone swish-and-spit solution, and pneumonitis (15 percent), which led to treatment discontinuation.



### Maintenance Therapy

A large retrospective study examined the categorical responses outcome of rituximab-naïve patients who were either observed or received maintenance rituximab.<sup>184</sup> Categorical responses improved after induction therapy in 42 percent of patients who received maintenance rituximab versus 10 percent in patients on observation. In addition, both progression-free survival (56.3 vs. 28.6 months) and overall survival (>120 vs. 116 months) were longer in patients who received maintenance rituximab. Improved progression-free survival was evident despite previous treatment status or induction with rituximab, either alone or in combination therapy. Best serum IgM response was also lower and hematocrit higher in those patients who received maintenance rituximab. Among patients who received maintenance rituximab therapy, an increased number of infectious events, predominantly grade 1 or 2 sinusitis and bronchitis were observed, along with lower serum IgA and IgG levels. A prospective study examining the role of maintenance rituximab was also initiated by the German STiL group.<sup>185</sup> In this study, patients received up to six cycles of bendamustine and rituximab, and responders were randomized to either observation or maintenance rituximab every 2 months for 2 years. Enrollment for this study is complete, and response outcome for maintenance rituximab therapy is awaited.

### HIGH-DOSE THERAPY AND STEM CELL TRANSPLANTATION

The European Bone Marrow Transplant Registry reported the largest experience for both autologous and allogeneic SCT in WM.<sup>186,187</sup> Among 158 WM patients receiving an autologous SCT, which included primarily relapsed or refractory patients, the 5-year progression-free and overall survival rate was 39.7 percent and 68.5 percent, respectively.<sup>186</sup> Nonrelapse mortality at 1 year was 3.8 percent. Chemorefractory disease and the number of prior lines of therapy at time of the autologous SCT were the most important prognostic factors for progression-free and overall survival. In the allogeneic SCT experience from the European Bone Marrow Transplant Registry, the long-term outcome of 86 WM patients was reported.<sup>187</sup> A total of 86 patients received allograft by either myeloablative or reduced-intensity conditioning. The median age of patients in this series was 49 years, and 47 patients had three or more previous lines of therapy. Eight patients failed prior autologous SCT. Fifty-nine patients (68.6 percent) had chemotherapy-sensitive disease at the time of allogeneic SCT. Nonrelapse mortality at 3 years was 33 percent for patients receiving a myeloablative transplant and 23 percent for those who received reduced-intensity conditioning. The overall response rate was 75.6 percent. The relapse rates at 3 years were 11 percent for myeloablative and 25 percent for reduced-intensity conditioning recipients. Five-year progression-free and overall survival for WM patients who received a myeloablative allogeneic SCT were 56 percent and 62 percent, respectively, and for patients who received reduced intensity conditioning were 49 percent and 64 percent, respectively. The occurrence of chronic graft-versus-host disease was associated with improved progression-free survival and suggested the existence of a clinically relevant graft-versus-WM effect in this study.

### ● RESPONSE CRITERIA IN WALDENSTRÖM MACROGLOBULINEMIA

Table 20-3 summarizes the response categories and criteria for progressive disease in WM based on the most recent consensus recommendations.<sup>188</sup> The term *overall response* is used to characterize all responses, including minor responses. Major responses only include partial, very good partial, and complete responses. The attainment of very good

**TABLE 20-3.** Summary of Consensus Response Criteria for Waldenström Macroglobulinemia<sup>187</sup>

|                            |      |                                                                                                                                                                                                                                                                                          |
|----------------------------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Complete response          | CR   | Absence of serum monoclonal IgM protein by immunofixation.<br>Normal serum IgM level.<br>Complete resolution of extramedullary disease, i.e., lymphadenopathy/splenomegaly if present at baseline.<br>Morphologically normal marrow aspirate and trephine biopsy.                        |
| Very good partial response | VGPR | Monoclonal IgM protein is detectable.<br>90% reduction in serum IgM level from baseline or normalization of serum IgM level.<br>Complete resolution of extramedullary disease, i.e., lymphadenopathy/splenomegaly if present at baseline.<br>No new signs or symptoms of active disease. |
| Partial response           | PR   | Monoclonal IgM protein is detectable.<br>≥50% but <90% reduction in serum IgM level from baseline.<br>Reduction in extramedullary disease, i.e., lymphadenopathy/splenomegaly if present at baseline.<br>No new signs or symptoms of active disease.                                     |
| Minor response             | MR   | Monoclonal IgM protein is detectable.<br>≥25% but <50% reduction in serum IgM level from baseline.<br>No new signs or symptoms of active disease.                                                                                                                                        |
| Stable disease             | SD   | Monoclonal IgM protein is detectable.<br><25% reduction and <25% increase in serum IgM level from baseline.<br>No progression in extramedullary disease, i.e., lymphadenopathy/splenomegaly.<br>No new signs or symptoms of active disease.                                              |
| Progressive disease        | PD   | >25% increase in serum IgM level from lowest nadir (requires confirmation) and/or progression in clinical features attributable to the disease.                                                                                                                                          |

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partial or complete responses is associated with improved progression-free survival.<sup>155,165,176,180,189</sup> Response assessments in WM rely primarily on serum IgM or IgM paraprotein levels, although complete responses require disappearance of the IgM monoclonal protein and resolution of marrow and/or extramedullary WM disease.<sup>189</sup> An important concern with the use of IgM as a surrogate marker of disease is that it can fluctuate, independent of tumor-cell killing with some agents. By way of example, rituximab can induce a flare in serum IgM levels, whereas everolimus, bortezomib, and ibrutinib can suppress IgM levels independent of tumor-cell killing in some patients, a finding referred to as IgM discordance.<sup>34,158,159,180,183,190</sup> Moreover, with selective B-cell-depleting agents such as rituximab and alemtuzumab, residual IgM-producing plasma cells are spared and continue to persist, thus potentially skewing

the relative response and assessment to treatment.<sup>191</sup> sCD27 levels have been investigated as an alternative surrogate marker in WM given their correlation with WM disease burden and may remain a faithful marker of disease in patients experiencing a rituximab-related IgM flare, as well as after plasmapheresis.<sup>192</sup> The use of quantitative allele-specific PCR assays to assess serial MYD88<sup>L265P</sup> burden in WM patients is also under investigation.<sup>36,38,193</sup>

## COURSE AND PROGNOSIS

WM typically presents as an indolent disease. The presence of 6q deletions may have prognostic significance, although this is disputed.<sup>30,194,195</sup> MYD88 WT status may be an inferior prognostic marker for survival.<sup>52</sup> Age is an important prognostic factor (>65 years)<sup>196–198</sup> but is influenced by comorbidities. Anemia that reflects both marrow involvement and the serum level of the IgM monoclonal protein (because of the impact of IgM on intravascular fluid retention) has emerged as a strong adverse prognostic factor, with hemoglobin levels of less than 9 to 12 g/dL associated with decreased survival in several series.<sup>196–198</sup> Other cytopenias

also may be significant predictors of survival.<sup>197</sup> Serum albumin levels also correlated with survival in WM patients in some studies, using multivariate analyses.<sup>197,199</sup> Elevated serum  $\beta_2$ -microglobulin levels (>3.0 to 3.5 g/dL) have also shown strong prognostic correlation in WM.<sup>198–201</sup> Several scoring systems have been proposed based on these analyses (Table 20–4), including the WM International Prognostic Scoring System (WM IPSS), which incorporates five adverse covariates: advanced age (>65 years), hemoglobin less than or equal to 11.5 g/dL, platelet count less than or equal to  $100 \times 10^9/L$ ,  $\beta_2$ -microglobulin greater than 3 mg/L, and serum monoclonal protein concentration greater than 7 g/dL.<sup>198</sup> Among 537 WM patients evaluated in the development of WM IPSS, low-risk patients (27 percent) presented with none or one of the adverse characteristics and advanced age, intermediate-risk patients (38 percent) with two adverse characteristics or only advanced age, and high-risk patients (35 percent) with more than two adverse characteristics. Five-year survival rates for these patients were 87 percent, 68 percent, and 36 percent, respectively. Importantly, the WM IPSS retained its prognostic significance in subgroups defined by age, treatment with alkylating agent and nucleoside analogues. Data from the Surveillance, Epidemiology, and End Results (SEER) database involving 7744 WM

**TABLE 20–4.** Prognostic Scoring Systems in Waldenström Macroglobulinemia

| Study                                                                                                           | Adverse Prognostic Factors      | Number of Groups                                 | Survival                |
|-----------------------------------------------------------------------------------------------------------------|---------------------------------|--------------------------------------------------|-------------------------|
| Gobbi and colleagues <sup>196</sup>                                                                             | Hgb <9 g/dL                     | 0–1 prognostic factors                           | Median: 48 months       |
|                                                                                                                 | Age >70 years                   | 2–4 prognostic factors                           | Median: 80 months       |
|                                                                                                                 | Weight loss                     |                                                  |                         |
|                                                                                                                 | Cryoglobulinemia                |                                                  |                         |
| Morel and colleagues <sup>197</sup>                                                                             | Age $\geq 65$ years             | 0–1 prognostic factors                           | 5 year: 87% of patients |
|                                                                                                                 | Albumin <4 g/dL                 | 2 prognostic factors                             | 5 year: 62%             |
|                                                                                                                 | Number of cytopenias:           | 3–4 prognostic factors                           | 5 year: 25%             |
|                                                                                                                 | Hgb <12 g/dL                    |                                                  |                         |
|                                                                                                                 | Platelets < $150 \times 10^9/L$ |                                                  |                         |
| Dhodapkar and colleagues <sup>200</sup>                                                                         | WBC < $4 \times 10^9/L$         |                                                  |                         |
|                                                                                                                 | $\beta_2M \geq 3$ g/dL          | $\beta_2M < 3$ mg/dL + Hgb $\geq 12$ g/dL        | 5 year: 87% of patients |
|                                                                                                                 | Hgb <12 g/dL                    | $\beta_2M < 3$ mg/dL + Hgb <12 g/dL              | 5 year: 63%             |
|                                                                                                                 | IgM <4 g/dL                     | $\beta_2M \geq 3$ mg/dL + IgM $\geq 4$ g/dL      | 5 year: 53%             |
|                                                                                                                 |                                 | $\beta_2M \geq 3$ mg/dL + IgM <4 g/dL            | 5 year: 21%             |
| Application of International Staging System Criteria for Myeloma to WM Dimopoulos and colleagues <sup>199</sup> | Albumin $\leq 3.5$ g/dL         | Albumin $\geq 3.5$ g/dL + $\beta_2M < 3.5$ mg/dL | Median: NR              |
|                                                                                                                 | $\beta_2M \geq 3.5$ mg/L        | Albumin $\leq 3.5$ g/dL + $\beta_2M < 3.5$ or    | Median: 116 months      |
|                                                                                                                 |                                 | $\beta_2M 3.5$ – $5.5$ mg/dL                     | Median: 54 months       |
|                                                                                                                 |                                 | $\beta_2M > 5.5$ mg/dL                           |                         |
| International Prognostic Scoring System for WM Morel and colleagues <sup>198</sup>                              | Age >65 year                    | 0–1 prognostic factors (excluding age)           | 5 year: 87% of patients |
|                                                                                                                 | Hgb <11.5 g/dL                  | 2 prognostic factors (or age >65 years)          | 5 year: 68%             |
|                                                                                                                 | Platelets < $100 \times 10^9/L$ | 3–5 prognostic factors                           | 5 year: 36%             |
|                                                                                                                 | $\beta_2M > 3$ mg/L             |                                                  |                         |
|                                                                                                                 | IgM >7 g/dL                     |                                                  |                         |

$\beta_2M$ ,  $\beta_2$ -microglobulin; Hgb, hemoglobin; NR, not reported; WBC, white blood cell count.

patients showed that the relative survival of WM patients has improved over time. Patients diagnosed during the years 2001 to 2010 had higher 5-year (78 percent vs. 67 percent) and 10-year (66 percent vs. 49 percent) relative survival rates versus patients diagnosed during the years 1980 to 2000.<sup>202</sup> A Greek study that included 345 patients with WM failed to show any overall or cause-specific survival improvement in recent years, although the study might have been too underpowered to detect any expected benefit.<sup>203</sup> A Swedish study of 1555 patients diagnosed with WM between 1980 and 2005 showed that the 5-year relative survival rate improved from 57 percent in 1980 to 1985 to 78 percent in 2001 to 2005.<sup>9</sup>

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## CHAPTER 21

# HEAVY-CHAIN DISEASE

Dietlind L. Wahner-Roedler and Robert A. Kyle

### SUMMARY

The heavy-chain diseases (HCDs) are B-cell lymphoplasmacytic proliferative disorders in which neoplastic cells produce monoclonal immunoglobulins (Igs) consisting of truncated heavy chains (HCs) without attached light chains. The complex abnormalities of HCD proteins and the usual lack of normal light chains are a result of several distinct gene alterations, including somatic mutations, deletions, and insertions. HCDs involving the three main immunoglobulin classes have been described:  $\alpha$ -HCD is the most common and has the most uniform presentation;  $\gamma$ - and  $\mu$ -HCDs have variable clinical presentations and histopathologic features. The diagnosis is established from immunofixation of serum, urine, or secretory fluids in the case of  $\alpha$ -HCD or by immunohistologic analysis of the proliferating lymphoplasmacytic cells in nonsecretory disease. Treatment of  $\alpha$ -HCD consists of antibiotics. If there is no response to antibiotics or if aggressive lymphoma is diagnosed, chemotherapy is indicated. Treatment of  $\gamma$ - and  $\mu$ -HCDs depends on the underlying clinicopathologic features rather than on the presence of the abnormal protein. Table 21–1 summarizes the features of the HCDs.

### ● $\gamma$ -HEAVY-CHAIN DISEASE DEFINITION AND HISTORY

$\gamma$ -Heavy-chain disease (HCD) is not a specific cytopathologic process; rather, it is a biochemical expression of a mutant B-cell clone. The disease should be considered a serologically determined entity with a variety of clinical and histopathologic features. It is defined by the recognition of monoclonal deleted gamma ( $\gamma$ ) chains devoid of light chains.<sup>1</sup>

The first case of  $\gamma$ -HCD was described in 1964 by Franklin and colleagues,<sup>2</sup> who observed a homogeneous band between  $\gamma$ - and  $\beta$ -globulin in an African American patient with generalized lymphadenopathy. Comparison of the proteins in the urine to those in the serum showed that they were the same, a suggestion of the presence of a low-molecular-weight serum  $\gamma$ -globulin, which then was shown to be a fragment of a  $\gamma$ -heavy chain (HC). Since this first description, more than 130 patients with  $\gamma$ -HCD have been described in the literature.<sup>3–5</sup>

### ● EPIDEMIOLOGY

$\gamma$ -HCD has been described throughout the world. Although initially  $\gamma$ -HCD was reported to occur equally in men and women,<sup>4</sup> there was a clear predominance of women in a more recent series of 23 patients.<sup>5</sup> The median age at diagnosis in that series was 68 years (range: 42 to 87 years).

**Acronyms and Abbreviations:** C<sub>H</sub>1 (2, 3, 4), constant region 1 (2, 3, 4); D, diversity; HC, heavy chain; HCD, heavy-chain disease; Ig, immunoglobulin; IPSID, immunoproliferative small intestinal disease; J, joining; V, variable.

### ● ETIOLOGY AND PATHOGENESIS

The etiology of  $\gamma$ -HCD is unknown.

### ● CLINICAL FEATURES

Originally,  $\gamma$ -HCD was considered to be a lymphomatous illness. However,  $\gamma$ -HCD has various clinical and pathologic features that can be divided into three broad categories, as described next.

#### DISSEMINATED LYMPHOPROLIFERATIVE DISEASE

Disseminated lymphoproliferative disease is present in most patients at the time of diagnosis, and various series have reported it in 57 percent to 66 percent of patients.<sup>4–6</sup> In two different series,<sup>4,5</sup> lymphadenopathy was present in 56 percent and 62 percent of patients, splenomegaly in 38 percent and 52 percent, and hepatomegaly in 8 percent and 37 percent at the time of diagnosis.

#### LOCALIZED PROLIFERATIVE DISEASE

In approximately 25 percent of patients, the lymphoproliferative process is localized, which may be extramedullary or may involve only the marrow.<sup>4,5</sup> Cutaneous involvement is the most frequently reported extramedullary presentation.<sup>4,5</sup> Patients may present with extramedullary plasmacytoma of the thyroid or parotid gland or an oropharyngeal mass<sup>5</sup> and hypertrophic spinal pachymeningitis.<sup>7</sup>

#### NO APPARENT PROLIFERATIVE DISEASE

A proliferative lymphoplasmacytic disease is not apparent in 9 percent to 17 percent of patients. In most of these patients, an underlying autoimmune disorder has been reported. Autoimmune disorders with or without underlying lymphoid proliferation include rheumatoid arthritis, autoimmune cytopenias, systemic lupus erythematosus, Sjögren syndrome, myasthenia gravis, thyroiditis, and vasculitis.<sup>5</sup>

### ● LABORATORY FEATURES

#### MOLECULAR BIOLOGY AND GENETICS

Most  $\gamma$ -HCD proteins are dimers of truncated HCs devoid of light chains. The molecular weight of the monomeric unit varies from 27,000 to 49,000. The length of the truncated  $\gamma$  chain varies, but usually it is one-half to three-fourths the length of the normal  $\gamma$  chain. Structural analysis of the defective monoclonal  $\gamma$ -HC of 23 patients with  $\gamma$ -HCD showed several characteristic features (Fig. 21–1). The proteins usually begin with a normal variable region. In most cases, this sequence is short and interrupted by a large deletion encompassing the remainder of the variable region, although four of the HCs shown in Fig. 21–1 appear to have retained most or all of their variable (V), diversity (D), and joining (J) sequences. In all  $\gamma$ -HCD proteins, the entire constant region 1 (C<sub>H</sub>1) domain is also deleted, with normal sequence beginning at the hinge or occasionally at the C<sub>H</sub>2 domain. C<sub>H</sub>1 is responsible for light-chain binding. In the absence of an associated light chain, the C<sub>H</sub>1 domain binds to heat shock protein 78 (HC-binding protein), and the HC undergoes proteasomal degradation rather than secretion.

In two cases of  $\gamma$ -HCD (OMM and RIV), genomic sequence data are available (Fig. 21–2). The presence of large deletions in the switch/C<sub>H</sub>1 regions of these two  $\gamma$ -HCD genes explains why the corresponding HCD proteins lack C<sub>H</sub>1. Because the normal C<sub>H</sub>1 acceptor splice site is deleted, the donor splice of the leader, or the J region, is

**TABLE 21–1.** Summary of Features of the Heavy-Chain Diseases

| Features                                 | Types of Heavy-Chain Diseases                     |                             |                                    |
|------------------------------------------|---------------------------------------------------|-----------------------------|------------------------------------|
|                                          | $\alpha$                                          | $\gamma$                    | $\mu$                              |
| Year described                           | 1968                                              | 1964                        | 1969                               |
| Incidence                                | Rare                                              | Very rare                   | Very rare                          |
| Age at diagnosis                         | Young adult (<30 years)                           | Older adult (60–70 years)   | Older adult (50–60 years)          |
| Demographics                             | Mediterranean region                              | Worldwide                   | Worldwide                          |
| Structurally abnormal monoclonal protein | IgA                                               | IgG                         | IgM                                |
| MGUS phase                               | No                                                | Rarely                      | Rarely                             |
| Urine monoclonal light chain             | No                                                | No                          | Yes                                |
| Urine abnormal heavy chain               | Small amounts                                     | Often present               | Infrequent                         |
| Sites involved                           | Small intestine, mesenteric lymph nodes           | Lymph nodes, marrow, spleen | Lymph nodes, marrow, liver, spleen |
| Pathology                                | Extranodal marginal zone lymphoma (MALT or IPSID) | Lymphoplasmacytoid lymphoma | Small lymphocytic lymphoma, CLL    |
| Associated diseases                      | Infection, malabsorption                          | Autoimmune diseases         | None                               |
| Therapy                                  | Antibiotics, chemotherapy                         | Chemotherapy                | Chemotherapy                       |

CLL, chronic lymphocytic leukemia; Ig, immunoglobulin; IPSID, immunoproliferative small intestinal disease; MALT, mucosa-associated lymphoid tissue; MGUS, monoclonal gammopathy of undetermined significance.

Adapted with permission from Witzig TE, Wahner-Roedler DL: Heavy chain disease. *Curr Treat Options Oncol* 2002 Jun;3(3):247-254.

spliced directly to the next available functional acceptor splice site at the beginning of the hinge or C<sub>H</sub>2 domain.

## SERUM AND URINE PROTEIN FINDINGS

The serum protein electrophoretic pattern is extremely variable. A monoclonal peak is detected in 60 percent to 86 percent of patients.<sup>4,5</sup> When present, it is most commonly in the  $\beta_1$  or  $\beta_2$  region. The median value of the monoclonal spike at diagnosis in 19 patients was 1.59 g/dL (range: 0.40 to 3.91 g/dL).<sup>5</sup> The diagnosis is established by immunofixation of the serum and a concentrated urine specimen. A modified immunoselection technique for the diagnosis of HCD has been described.<sup>8</sup> In one case of  $\gamma$ -HCD, low concentrations of free HCs in serum were detected by capillary zone electrophoresis coupled with immunosubtraction.<sup>9</sup> A HC/light-chain assay to identify truncated immunoglobulin (Ig) HCs was used on serum samples from 15 patients with known  $\gamma$ -HCD. By this method, 20 percent of these patients were shown to also have small amounts of monoclonal free light chains.<sup>10</sup> The amount of HCD protein in the urine usually is small (<1 g/24 hours) but may reach 20 g/24 hours. An occasional patient with Bence Jones proteinuria has been described.<sup>5</sup>

No standard has been established to identify the subclass of the HC fragment. In reported cases in which the HC fragment subclass has been studied, different methods have been used, ranging from Ouchterlony in the earlier cases to indirect immunofluorescence staining, immunoblotting, amino acid sequence, immunoselection, and enzyme-linked immunosorbent assay.<sup>11</sup> IgG subclass distribution shows a lower-than-expected incidence of IgG<sub>2</sub>. The most common subclass is IgG<sub>1</sub>, which occurs in 65 percent of cases. IgG<sub>3</sub> has been identified in 27 percent of patients, IgG<sub>4</sub> in 5 percent, and IgG<sub>2</sub> in 3 percent.<sup>4</sup> Although biclonal gammopathy has been reported in 1 percent to 8 percent of all patients with serum monoclonal components, the association between  $\gamma$ -HCD

and another monoclonal protein is much higher. In a series of 23 patients with  $\gamma$ -HCD, 7 percent had an IgM- $\lambda$  intact monoclonal immunoglobulin.<sup>5</sup> No association between  $\gamma$ -HCD and monoclonal IgA has been described, although the IgG-IgA association was the most frequent in several series of biclonal gammopathies. One patient described in the literature was unique in that the serum contained two deleted  $\gamma$  chains of different subclasses (IgG<sub>1</sub> and IgG<sub>2</sub>).<sup>12</sup>

## HEMATOLOGIC ABNORMALITIES

Anemia is frequent. It is usually normochromic, normocytic, and moderate. Coombs-positive autoimmune hemolytic anemia has been reported in several cases and may be associated with thrombocytopenia (Evans syndrome). The total and differential leukocyte counts are usually normal. Lymphocytosis may occur, and an occasional patient presents with chronic lymphocytic leukemia. In some cases, rare plasmacytoid lymphocytes or plasma cells have been noted in the blood. Plasma cell leukemia has been reported in two patients.<sup>13,14</sup>

Marrow aspirates and biopsy specimens often show an increase of plasma cells, lymphocytes, or plasmacytoid lymphocytes, similar to the marrow findings in Waldenström macroglobulinemia. The typical marrow features of myeloma or chronic lymphocytic leukemia are rare. An unusual concurrence of T-cell large granular lymphocytic leukemia with  $\gamma$ -HCD has been reported.<sup>15,15a,15b</sup> Marrow changes consistent with a myeloproliferative neoplasm have been noted in a few patients.<sup>5</sup>

## OTHER FEATURES

Bone lesions are rare in  $\gamma$ -HCD. Cytogenetic studies have seldom been reported. No unique abnormalities or characteristics of lymphoma have been found.



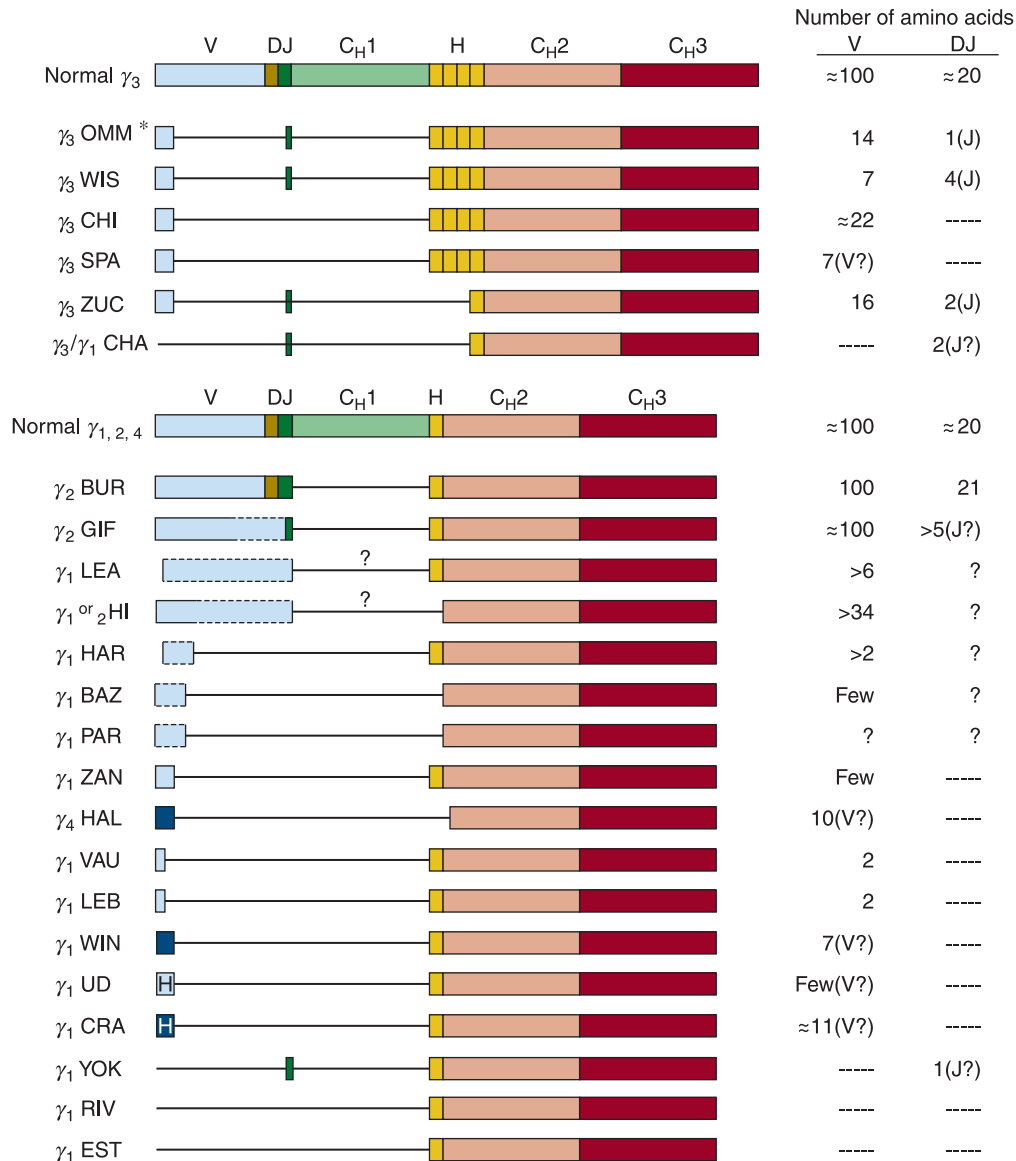


**Figure 21-1.** Structure of various deleted  $\gamma$ -heavy-chain disease (HCD) proteins compared with that of normal chains. \*Structure shown is a primary synthetic product synthesized by the HCD cells. Serum protein was modified after synthesis and did not contain any amino acids before the hinge. [H] indicates heterogeneous amino acid sequences; [H], unusual and heterogeneous amino acid sequences; [H], unusual amino acid sequences; boxes, coding regions; lines, deletions; dashed lines, likely structures for which sequence data are missing; ?, probable missing domain based on molecular weight and partial protein structure analysis; V, variable region; D, diversity segment; J, joining region; H, hinge region;  $C_H1$ ,  $C_H2$ ,  $C_H3$ , constant regions of heavy chains. OMM,<sup>66</sup> WIS,<sup>67</sup> CHI,<sup>68</sup> SPA,<sup>69</sup> ZUC,<sup>70</sup> CHA,<sup>71</sup> BUR,<sup>72</sup> GIF,<sup>73</sup> LEA,<sup>74</sup> HI,<sup>75</sup> HAR,<sup>76</sup> BAZ,<sup>76</sup> PAR,<sup>77</sup> ZAN,<sup>78</sup> HAL,<sup>79</sup> VAU,<sup>80</sup> LEB,<sup>80</sup> WIN,<sup>81</sup> UD,<sup>82</sup> CRA,<sup>83</sup> YOK,<sup>84</sup> RIV,<sup>85</sup> EST.<sup>86</sup>

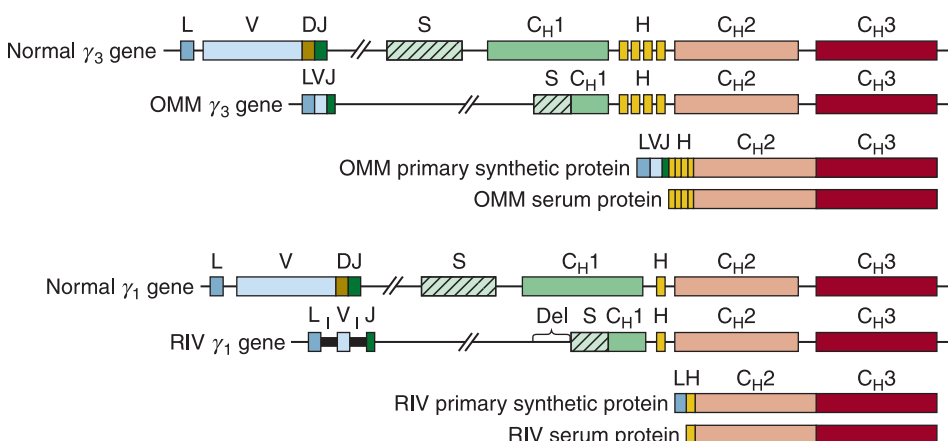


**Figure 21-2.** Structure of two genes coding for  $\gamma$ -heavy-chain disease proteins compared with that of normal  $\gamma_3$  and  $\gamma_1$  genes. Boxes indicate coding regions; [H], switch region; [H], inserted noncoding sequence; lines, intervening (noncoding) sequences; L, leader region; V, variable region; D, diversity segment; J, joining region; S, switch region; H, hinge region;  $C_H1$ ,  $C_H2$ ,  $C_H3$ , constant regions of heavy chains; L, inserted sequence; Del, deleted sequence. OMM,<sup>66</sup> RIV.<sup>85</sup>

## HISTOPATHOLOGY

In contrast to  $\alpha$ -HCD,  $\gamma$ -HCD has no consistent morphologic pattern, and a variety of underlying lymphoproliferative disorders have been described.<sup>16</sup> The most frequent histopathologic finding is a pleomorphic malignant lymphoplasmacytic proliferation in marrow and lymph nodes. These lymphocytoid plasma cells express pan-B-cell markers and cytoplasmic  $\gamma$ -HC without light chains and are negative for CD5 and CD10.<sup>17</sup>

Lymphoma without any consistent morphologic type was diagnosed in 18 (38 percent) of 47 patients in whom lymph nodes were examined. A lymphoplasmacytic proliferation was present in 36 percent and hyperplastic nodes and plasmacytoma in 11 percent each; there was one case of Hodgkin lymphoma and one of probable Hodgkin lymphoma.<sup>6</sup> Plasmacytic infiltration may be found in the salivary glands or thyroid.<sup>4,5,18</sup>

## DIFFERENTIAL DIAGNOSIS

All patients presenting with a lymphoplasmacytic proliferative disorder should be evaluated for  $\gamma$ -HCD.

## THERAPY

Because  $\gamma$ -HCD is a heterogeneous condition, the choice of therapy depends on the clinical findings. In an asymptomatic patient with a monoclonal  $\gamma$ -HC, no therapy is indicated. Any associated autoimmune disease should be managed with standard therapy for that specific disease type. In symptomatic patients with a low-grade lymphoplasmacytic malignancy, a trial of chlorambucil may be beneficial. Melphalan and prednisone can be used if the proliferation is predominantly plasmacytic. A trial of cyclophosphamide, vincristine, and prednisone with or without doxorubicin is reasonable for patients with evidence of a progressive lymphoplasmacytic proliferative process or high-grade lymphoma. One patient achieved a complete response after six courses of fludarabine.<sup>19</sup> Successful treatment of  $\gamma$ -HCD with low-dose etoposide has been reported.<sup>20</sup> CD20 expression has been analyzed in only seven cases and was detected in six of the seven, including one in which CD20 expression appeared transient.<sup>5,21–23</sup> Rituximab monotherapy was given in two cases, resulting in clinical responses in both.<sup>5,21</sup> In two other cases, a combination of rituximab with chemotherapy had an antitumor effect in lymphoplasmacytic-type  $\gamma$ -HCD.<sup>22,24</sup> An adolescent with stage IV extranodal marginal zone lymphoma with  $\gamma$ -HCD achieved complete response with treatment consisting of bendamustine plus rituximab.<sup>24a</sup> In localized extramedullary plasmacytomas treated with radiation<sup>4</sup> or surgical removal (or both), complete clinical and serologic remission has been achieved.

## COURSE AND PROGNOSIS

The clinical course of  $\gamma$ -HCD is extremely variable and ranges from an asymptomatic, benign, or transient process to a rapidly progressive neoplasm leading to death within a few weeks. Patients with the features of only a monoclonal gammopathy have remained clinically well for 2 to 7 years of followup.<sup>5,25</sup> Spontaneous disappearance of the  $\gamma$ -HCD protein has been reported.<sup>4</sup> The median duration of survival in a series of 23 patients was 7.4 years (range: 1 month to more than 21 years).<sup>5</sup>

The amount of serum  $\gamma$ -HCD protein usually parallels the severity of the associated malignant process. Disappearance of the monoclonal component from serum, and urine associated with apparent complete response has been induced by chemotherapy,<sup>19</sup> radiotherapy,<sup>4</sup> or surgical removal of a localized process. In some instances, however, the  $\gamma$ -HCD

protein does not vary in parallel with the associated process, and relapse can occur without the reappearance of the pathologic protein.<sup>4</sup>

## $\alpha$ -HEAVY-CHAIN DISEASE DEFINITION AND HISTORY

$\alpha$ -HCD is a proliferative disorder of B-lymphoid cells involving the IgA secretory immune system, especially the gastrointestinal tract. It is defined by the recognition of internally deleted monoclonal  $\alpha$  chains devoid of light chains.

In the first case description of  $\alpha$ -HCD in 1968 by Seligman and colleagues,<sup>26</sup> an Arab woman had severe malabsorption resulting from a lymphoplasmacytic infiltrate in the small bowel. Since then, more than 400 cases have been reported.

## EPIDEMIOLOGY

The majority of reported cases have been from northern Africa, Israel, and surrounding Middle Eastern countries. A common variable for patients with  $\alpha$ -HCD is a low socioeconomic status. In a study of the distribution of monoclonal gammopathies in Tunisia published in 1990, 17 percent of 198 cases were attributed to  $\alpha$ -HCD.<sup>27</sup> In a later study of 270 cases observed between 1992 and 2000 at the university hospital of Sfax in Tunisia, only 2.2 percent were attributed to  $\alpha$ -HCD,<sup>28</sup> a finding that might be partially explained by improved socioeconomic conditions. Similarly, a persistent decrease in the incidence of immunoproliferative small intestinal disease (IPSID) since 1986 as a result of improving sanitation has been reported from Iran<sup>29</sup> and Greece.<sup>30</sup>  $\alpha$ -HCD has a predilection for young adults. The prevalence of the disease is slightly higher in males than in females.

## ETIOLOGY AND PATHOGENESIS

The cause of  $\alpha$ -HCD is unknown. The disease might be considered a model showing the complex interactions of the environment with genetic factors and the infection-immunity-cancer interrelationships originating from the same proliferating clone. Although the mechanisms leading to the development of a clonal population synthesizing the structurally abnormal IgA are still speculative, the lymphoplasmacytic infiltration of the intestinal mucosa is likely a response of the alimentary tract immune system to protracted luminal antigenic stimulation. A causal relationship between infection and pathogenesis is supported by the observation that  $\alpha$ -HCD can respond to broad-spectrum antibiotics. Using molecular strategies, *Campylobacter jejuni* was detected in five of seven patients with  $\alpha$ -HCD.<sup>31</sup> However, no specific microorganism has been found in other clinical studies. The putative agent may be present only at the onset of the disease and absent at diagnosis.

## CLINICAL FEATURES

In most cases, patients who have  $\alpha$ -HCD present with the digestive form. The disease is characterized by malabsorption manifested by diarrhea, weight loss, and abdominal pain. Ascites, tetany, edema, and clubbing may be present. Hepatosplenomegaly and peripheral lymphadenopathy are infrequent. Fever is uncommon. Amenorrhea, alopecia, and growth retardation in children and adolescents correlate with the duration and the severity of the malabsorptive process.  $\alpha$ -HCD may be confined to the respiratory tract, but this is extremely rare, as is a lymphomatous form characterized by generalized lymphadenopathy.  $\alpha$ -HCD has been reported in a patient with a goiter from a plasmacytoma of the thyroid<sup>32</sup>

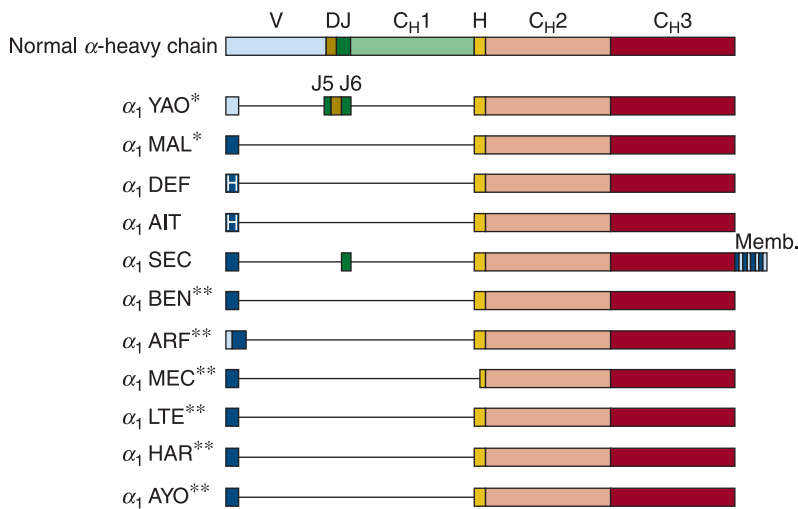


**Figure 21-3.** Structure of various  $\alpha$ -heavy-chain disease (HCD) proteins compared with that of normal chain. \*Structures shown are primary synthetic products synthesized by the HCD cells. Serum proteins were modified after synthesis and did not contain any amino acids before the hinge. \*\*Structures shown are deduced amino acid sequences determined by complementary DNA sequencing. [H] indicates unusual and heterogeneous amino acid sequences; [■], unusual amino acid sequences; boxes, coding regions; lines, deletion; V, variable region; D, diversity segment; J, joining region; H, hinge region;  $C_H1$ ,  $C_H2$ ,  $C_H3$ , constant regions of heavy chains; Memb., membrane exon. YAO,<sup>87</sup> MAL,<sup>88</sup> DEF,<sup>89</sup> AIT,<sup>90</sup> SEC,<sup>91</sup> BEN,<sup>92</sup> ARF,<sup>92</sup> MEC,<sup>92</sup> LTE,<sup>92</sup> HAR,<sup>92</sup> AYO.<sup>92</sup>

and in a patient with polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin lesions (POEMS).<sup>33</sup>

## LABORATORY FEATURES

### MOLECULAR BIOLOGY AND GENETICS

Most  $\alpha$ -HCD proteins consist of multiple polymers. The molecular weight of the basic monomeric unit varies from 29,000 to 34,000. The length of the basic polypeptide subunit differs from patient to patient and in most instances is between one-half and three-fourths that of a normal  $\alpha$  chain. Sequence data are available for several  $\alpha$ -HCD proteins (Fig. 21-3). In all cases of  $\alpha$ -HCD studied, the  $\alpha$ -HCD protein belonged to the  $\alpha_1$  subclass. Common features of the defective  $\alpha$  chain include deleted V regions, missing  $C_H1$  domains, and the absence of light chains. Most of the proteins have short, non-Ig-related sequences of unknown origin at the amino terminus. The complete sequences of the genes encoding three  $\alpha$ -HCD proteins are shown in Fig. 21-4. These three genes show striking similarity in their position and extent of the two main deletions, which encompass sequences in the V/J and the switch/ $C_H1$  region.

### SERUM, URINE, AND INTESTINAL FLUID PROTEIN FINDINGS

In contrast to other monoclonal gammopathies, the characteristic sharp spike of a monoclonal gammopathy is not found on serum protein electrophoresis in  $\alpha$ -HCD. In about half of cases, an abnormal broad band is found in the  $\alpha_2$ - or  $\beta$ -globulin region, which is probably related to polymerization of the  $\alpha$  chains. In the other half of cases, serum protein electrophoresis shows no evidence of an abnormal protein. Identification of the  $\alpha$ -HCD protein depends on immunoselection or immunofixation. The pathologic protein may easily escape detection by immunoelectrophoresis when its serum concentration is low. In most patients, the  $\alpha$ -HCD protein can be found in the serum. During the course of the disease, the progressive diminution of mature plasma cells and their replacement by immature immunoblasts likely is followed by a progressive decrease in the serum concentration of  $\alpha$ -HCD protein.  $\alpha$ -HCD protein hyposecretion also may be found during the early stage of the disease.

In most cases, the  $\alpha$ -HCD protein also is found in the jejunal secretions.<sup>34</sup>  $\alpha$ -HCD protein has been found in the intestinal or gastric fluid in a few cases when it was undetectable in serum and urine. The

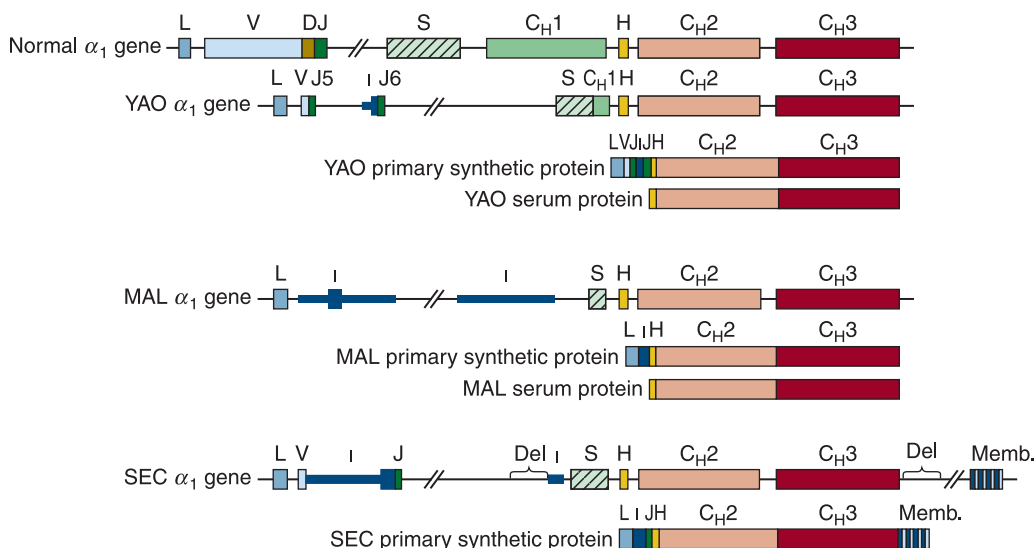


**Figure 21-4.** Structure of three genes coding for different  $\alpha_1$ -heavy-chain disease proteins compared with that of normal  $\alpha_1$  gene. Boxes indicate coding regions; [■], switch region; [■], inserted coding sequence; [■], inserted noncoding sequence; lines, intervening (noncoding) sequences; L, leader region; V, variable region; D, diversity segment; J, joining region; S, switch region; H, hinge region;  $C_H1$ ,  $C_H2$ ,  $C_H3$ , constant regions of heavy chains; I, inserted sequence; Del, deleted sequence; Memb., membrane exon. YAO,<sup>87</sup> MAL,<sup>88</sup> SEC.<sup>91</sup>



concentration of  $\alpha$ -HCD protein in the urine is low. Bence Jones proteinuria has never been documented.

Synthesis of the  $\alpha$ -HCD protein by the proliferating cells has been demonstrated by immunohistochemical or immunocytochemical methods and by biosynthesis studies *in vitro*.<sup>35</sup> These techniques are helpful in the recognition of nonsecreting forms of  $\alpha$ -HCD.

## HEMATOLOGIC AND METABOLIC ABNORMALITIES

Mild-to-moderate anemia is often found. Hypokalemia, hypocalcemia, hypomagnesemia, and hypoalbuminemia are common. The intestinal isoenzyme fraction of the alkaline phosphatase level may be increased. Results of tests to indicate malabsorption are usually positive.

## IMAGING PROCEDURES

Abnormal radiographic findings of the small intestine include hypertrophic and pseudopolypoid mucosal folds, occasionally associated with strictures and filling defects. The extent of the disease should be evaluated with computed tomography.

## ENDOSCOPY

$\alpha$ -HCD intestinal lesions nearly always affect the duodenum and jejunum, making endoscopy with biopsy a useful tool in the evaluation of patients in whom  $\alpha$ -HCD is suspected. Several endoscopic patterns have been defined. The infiltrated pattern is the most specific, followed by the nodular pattern. Other primary lesions (ulcerations, mosaic pattern, and mucosal fold thickening alone) are nonspecific.

## HISTOPATHOLOGY

In the digestive form of  $\alpha$ -HCD, the proliferation involves at least the proximal half of the small intestine and adjacent mesenteric lymph nodes. The whole length of the small bowel, the gastric, and the colorectal mucosae that belong to the IgA secretory system may be involved.

The disease progresses in three histopathologic stages according to Galian and colleagues.<sup>36</sup> In stage A, a mature plasmacytic or lymphoplasmacytic infiltration of the mucosal lamina propria is noted. Villous atrophy is variable. Stage B is characterized by the presence of atypical plasmacytic or lymphoplasmacytic cells and more or less atypical immunoblast-like cells extending at least to the submucosa. Subtotal or total villous atrophy is present. Stage C corresponds to an immunoblastic lymphoma. Similar to the changes described in the small intestine, three histologic stages (A, B, C) have been described in the mesenteric lymph nodes. Involvement of liver, spleen, and peripheral lymph nodes is uncommon. The histologic lesions may progress at any given site from stage A to stage B or from stage B to stage C. However, different stages can be found at the same time in different organs or even at different sites in the same organ. Thus, accurate pathologic staging of  $\alpha$ -HCD requires a laparotomy with sampling of multiple sites in all patients with  $\alpha$ -HCD in whom no stage C lesions are found on peroral biopsy. This recommendation is based on the observation that mesenteric lymph nodes may harbor malignant lymphoma when the intestinal mucosa reveals only a benign-appearing cellular infiltrate that one might be tempted to treat with antibiotics alone.<sup>37</sup> A staging system based on anatomical spread of the disease, which is complementary to the Galian staging system, has been published<sup>38</sup>; however, most physicians use the Galian staging system for determining prognosis and therapeutic strategies.

In the past, confusion existed over whether Mediterranean lymphoma and  $\alpha$ -HCD were different conditions. In 1976, a consensus

panel concluded that  $\alpha$ -HCD and Mediterranean lymphoma constitute a spectrum of disease, and the term *immunoproliferative small intestinal disease* (IPSID) came into use. This term is applied to small intestinal lesions whose pathologic features are identical to those of  $\alpha$ -HCD, regardless of the type of immunoglobulin synthesized.<sup>34,39</sup> The pattern of  $\alpha$ -HCD pathologic lesions often includes clear lymphoepithelial lesions composed of centrocytic-like cells. This indicates that  $\alpha$ -HCD can be considered a subtype of lymphoma arising from mucosa-associated lymph node tissue.<sup>40</sup> The pathologic changes in the few cases of the respiratory form of  $\alpha$ -HCD are poorly documented. In a case of the lymph node or lymphomatous form, lymph node biopsy showed diffuse plasmacytic lymphoma.

## CYTOGENETICS

Cytogenetic abnormalities have been found in the lymphoid cells of patients with  $\alpha$ -HCD. The clonal proliferation in this disease appears to be associated with frequent alterations of chromosome 14 at band q32 resulting from translocations that differ from those observed in the vast majority of other lymphomas. Abnormal karyotypes were reported in three of four patients.<sup>41</sup> Two patients had a rearrangement of 14q32 resulting from a t(9;14)(p11;q32) and a t(2;14)(p12;q32). Cloning and sequencing of the der(14) breakpoint of a chromosome translocation involving the 14q32 Ig locus in one of these patients suggested that the translocation originated from a local pairing of the two chromosomes, 9 and 14.<sup>42</sup> One case showed complex rearrangements, including t(5;9). No abnormalities were found in the intestinal tumor of the fourth case with immunoblastic lymphoma.

## DIFFERENTIAL DIAGNOSIS

The digestive form of  $\alpha$ -HCD must be differentiated from lymphoma, although this is an uncommon diagnosis in the age range typical of  $\alpha$ -HCD. Other causes of malabsorption need to be considered, especially celiac disease. Enteric presentation of  $\gamma$ -HCD, variable immunodeficiency, and acquired immunodeficiency syndrome with clinicopathologic features simulating IPSID should be excluded.

## THERAPY

Patients with stage A lesions limited to the bowel and to the mesenteric lymph nodes should be treated initially with oral antibiotics. In the absence of a documented parasite, tetracycline, metronidazole, or ampicillin is appropriate. Patients with stage B or C lesions or stage A lesions without improvement after a 6-month course of antibiotic treatment should be given chemotherapy. The treatment regimens are those commonly used to treat lymphoma. There have been few controlled clinical trials. In a prospective randomized study, a doxorubicin-based regimen (cyclophosphamide, doxorubicin hydrochloride, vincristine, and prednisone) provided a higher response rate than a non-doxorubicin-containing protocol (cyclophosphamide, vincristine, procarbazine, and prednisone) or total abdominal irradiation.<sup>43</sup> Similar results were noted in a retrospective study.<sup>44</sup> Good results have been reported with cyclophosphamide, doxorubicin, teniposide, and prednisone, sometimes alternating with bleomycin, vinblastine, and doxorubicin<sup>45</sup> and with cyclophosphamide, epidoxorubicin, vincristine, prednisolone, ifosfamide, methotrexate, etoposide (VP-16), and dexamethasone.<sup>46</sup> Surgical resection should be considered for focal or bulky transmural lymphomatous tumors and extramedullary plasmacytoma. Autologous hematopoietic stem cell transplantation has been recommended for patients with advanced or refractory disease,<sup>34</sup> but to our knowledge

there are no reports in the literature demonstrating the usefulness of this approach. Previous trials have not incorporated immunotherapy with rituximab, an anti-CD20 monoclonal antibody, in the management of IPSID. As expected, the centrocyte-like cells are CD20 positive, but the plasma cells are not. In light of the extreme plasma cell differentiation and the plasmacytic nature of large-cell IPSID lymphoma, there is interest in investigating the value of treating patients with IPSID with the novel agents (thalidomide, lenalidomide, bortezomib, pomalidomide, or carfilzomib). Transformation of IPSID to diffuse large B-cell lymphoma despite treatment with rituximab and an extended course of antibiotics, which then resulted in remission of the diffuse B-cell lymphoma with systemic chemotherapy after treatment with six cycles of rituximab, cyclophosphamide, vincristine, doxorubicin, prednisone, and lenalidomide, has been reported.<sup>46a</sup>

## COURSE AND PROGNOSIS

The course of  $\alpha$ -HCD is variable but generally progressive in the absence of therapy. Followup should include a periodic search for  $\alpha$ -HCD protein in serum and urine and, if negative, in the intestinal secretions. Bowel radiography, ultrasonography, and esophagogastroduodenal endoscopy should be performed. A second-look laparotomy may be necessary.<sup>34</sup> Relapses may occur after treatment at any stage of the disease. The long-term prognosis for patients with  $\alpha$ -HCD remains imprecise because of the lack of large series with prolonged followup. In a small prospective Tunisian study,<sup>45</sup> including eight patients with stage A disease and 15 with stages B and C, the survival of the total group was 90 percent at 2 years. A series from Turkey<sup>47</sup> reported 5-year treatment results of 23 patients with IPSID, including five with secretion of  $\alpha$  chains. In patients with stage A disease, tetracycline yielded a 71 percent complete response. The 5-year overall survival rate for the entire group was 70 percent. However, the median overall survival for three patients with immunoblastic lymphoma was only 7 months.

Thirteen patients were studied who had IPSID associated with  $\alpha$ -HCD.<sup>48</sup> Six patients, two with high-grade lymphoma and four with low-grade disease, received chemotherapy or radiotherapy or both. One patient died at 76 months, and five were alive at an average of 92 months. Five patients with low-grade disease received conservative therapy (antibiotics and in some cases prednisone). All five patients were alive at an average of 40 months after presentation. Three of these five patients achieved remission at 5, 6, and 27 months. Two of the five patients had persistent disease at 20 and 25 months. Two patients did not receive treatment and died of high-grade lymphoma.

Another study<sup>49</sup> described six patients who had  $\alpha$ -HCD with lymphoma. All patients responded poorly to chemotherapy; the median duration of survival was 10.5 months.

A subsequent study<sup>50</sup> described 12 patients with secretory and nonsecretory IPSID. Six patients presented with stage A disease. Four patients responded to antibiotic or glucocorticoid therapy. In two patients, stage A disease evolved into stage C. Three patients presented with stage B disease. Two of these patients responded completely to chemotherapy, and the third refused treatment and died after 16 months. Three patients with stage C disease at diagnosis received aggressive combination chemotherapy and remained in complete remission with a median followup of 2.2 years.

Preliminary results suggest that flow cytometric analysis of S-phase fraction<sup>51</sup> and certain immunomarkers, such as syndecan, Bcl6, and p53,<sup>52</sup> may be useful prognostic indicators in the clinical management of patients with IPSID. Patients with a poor prognosis have a higher fraction of cells in S phase, lower syndecan-1 expression, and higher Bcl6 expression than those with a good prognosis.

## $\mu$ -HEAVY-CHAIN DISEASE DEFINITION AND HISTORY

$\mu$ -HCD is a proliferative disorder of B lymphocytes defined by the recognition of monoclonal deleted  $\mu$ -HCs. In the first reported case, in 1969 by Forte and colleagues,<sup>53</sup> the patient had chronic lymphocytic leukemia. Since then, approximately 34 additional cases have been reported.<sup>54–57</sup>

## EPIDEMIOLOGY

$\mu$ -HCD is extremely rare. In a series of 27 patients, the majority were white (76 percent) and male (55 percent). The median age at diagnosis in 27 patients was 57.5 years (range: 15 to 80 years).<sup>54</sup>

## ETIOLOGY AND PATHOGENESIS

The cause of  $\mu$ -HCD is unknown.

## CLINICAL FEATURES

The most common presenting symptoms of patients with  $\mu$ -HCD are those of a lymphoproliferative malignancy. An associated lymphoplasma-cytic proliferative disorder was noted in 22 of 27 patients at some time during the disease and designated as chronic lymphocytic leukemia, lymphoma, Waldenström macroglobulinemia, or myeloma.<sup>54</sup>  $\mu$ -HCD protein has been described in one patient each with systemic lupus erythematosus, hepatic cirrhosis, hepatosplenomegaly with ascites, pulmonary infection, splenomegaly with pancytopenia,<sup>54</sup> and myelodysplasia.<sup>55</sup> Three cases of  $\mu$ -HCD associated with amyloidosis have been reported.<sup>58</sup>

Splenomegaly and hepatomegaly are common in  $\mu$ -HCD and were noted in 21 of 22 and 15 of 21 patients, respectively.<sup>54</sup> Peripheral lymphadenopathy was described in 10 of 25 patients.<sup>54</sup>

## LABORATORY FEATURES

### MOLECULAR BIOLOGY AND GENETICS

The molecular weight of the  $\mu$ -HCD protein determined in eight patients ranged from 26,500 to 158,000. The higher molecular weights are thought to be the result of polymerization of the  $\mu$ -chain fragments. The  $\mu$ -HCD fragments from six patients were subjected to detailed chemical analysis. Figure 21–5 depicts the structure of these six  $\mu$ -HCD proteins compared with that of normal  $\mu$ -HC. The  $V_H$  domain is absent in all cases. The normal sequence began with  $C_H1$  in three cases,  $C_H2$  in two cases, and  $C_H3$  in one case. There are sequence data for only 1 gene coding for a  $\mu$ -HCD protein (Fig. 21–6).

### SERUM AND URINE PROTEIN FINDINGS

A monoclonal spike was found on serum protein electrophoresis in less than half of a series of patients with  $\mu$ -HCD (eight of 19).<sup>54</sup> The diagnosis of  $\mu$ -HCD is made by documentation of the abnormal HC. Immunofixation of both serum and urine should be done. When these procedures yield ambiguous results, two-dimensional gel electrophoresis is a useful additional tool. The combination of capillary immunotyping electrophoresis and high-resolution two-dimensional electrophoresis has been used successfully for the detection of  $\mu$ -HCD in one patient,<sup>59</sup> whereas in another capillary zone electrophoresis failed to detect the  $\mu$ -HCD protein.<sup>60</sup> Three of 33 reported patients with  $\mu$ -HCD had a

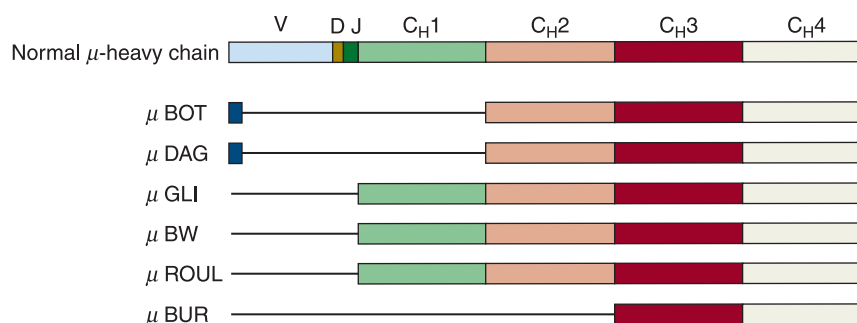


**Figure 21-5.** Structure of various deleted  $\mu$ -heavy-chain disease proteins compared with that of normal chain. ■, indicates unusual amino acid sequences; boxes, coding regions; lines, deletions; V, variable region; D, diversity segment; J, joining region;  $C_H1$ ,  $C_H2$ ,  $C_H3$ ,  $C_H4$ , constant regions of heavy chains. BOT,<sup>93</sup> DAG,<sup>94</sup> GLI,<sup>95</sup> BW,<sup>96</sup> ROUL,<sup>97</sup> BUR.<sup>98</sup>

biclonal gammopathy. Hypogammaglobulinemia was noted in 10 of 22 patients.<sup>54</sup> Hypergammaglobulinemia with a polyclonal pattern in the  $\gamma$ -globulin fraction was described in one case.<sup>55</sup> In contrast to  $\gamma$ - and  $\alpha$ -HCD, in which there usually is no detectable monoclonal light chain in the serum and urine, Bence Jones proteinuria was found in more than half the cases of  $\mu$ -HCD (14 of 22 patients).<sup>54</sup>  $\mu$ -HCD protein was found in the urine of only two patients.<sup>54</sup> Three cases of nonsecretory  $\mu$ -HCD have been reported.<sup>61-63</sup>  $\mu$ -HCs were documented by immunofluorescence on the cell surface of proliferating lymphocytes in one case and in marrow plasma cells of two others.

## HEMATOLOGIC ABNORMALITIES

Anemia is frequent, but lymphocytosis and thrombocytopenia are uncommon. One patient had a positive direct antiglobulin test.<sup>55</sup> Examination of the marrow usually shows an increase in lymphocytes, plasma cells, or plasmacytoid lymphocytes. Plasmacytosis was noted in 18 of 20 cases; in 13 of these, vacuolated plasma cells were found.<sup>54</sup> The presence of vacuolated plasma cells in the marrow of a patient with a lymphoplasmacytic proliferative disorder should always suggest the possibility of  $\mu$ -HCD.

## OTHER FEATURES

Lytic bone lesions were described in three of 15 patients,<sup>54</sup> and osteoporosis was mentioned in three others. No cytogenetic studies have been reported.

## HISTOPATHOLOGY

In a literature review that included 27 documented cases of  $\mu$ -HCD, 22 patients (81 percent) had an associated lymphoplasmacytic proliferative disorder designated as chronic lymphocytic leukemia, lymphoma, Waldenström macroglobulinemia, or myeloma.<sup>54</sup>

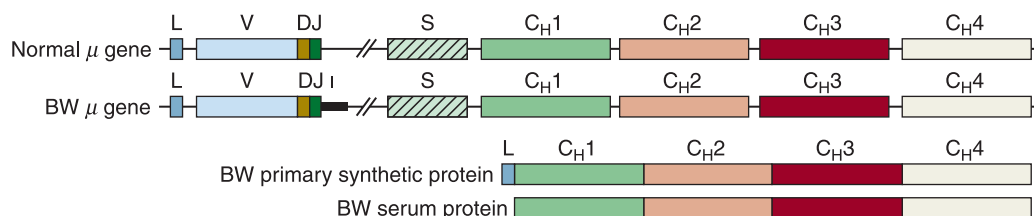


**Figure 21-6.** Structure of a gene coding for a  $\mu$ -heavy-chain disease protein compared with that of normal  $\mu$  gene. Boxes indicate coding regions; ▨, switch region; ■, inserted noncoding sequence; L, leader region; V, variable region; D, diversity segment; J, joining region; S, switch region;  $C_H1$ ,  $C_H2$ ,  $C_H3$ ,  $C_H4$ , constant regions of heavy chains; I, inserted sequence. BW.<sup>96</sup>

## DIFFERENTIAL DIAGNOSIS

The differential diagnosis of  $\mu$ -HCD includes all lymphoplasmacytic proliferative disorders. Without a suspicion for the disease,  $\mu$ -HCD is difficult to diagnose. The finding of Bence Jones proteinuria in a patient with a lymphoproliferative disorder and vacuolated plasma cells in the marrow deserves further investigation for possible  $\mu$ -HCD.

## THERAPY

There is no specific therapy for  $\mu$ -HCD. The finding of a  $\mu$ -HCD protein in the serum of an apparently normal patient should be considered to represent monoclonal gammopathy of undetermined significance, and the patient should be followed closely for the development of a symptomatic lymphoplasmacytic proliferative disorder. Once this develops, chemotherapy is indicated. Various agents have been used. Initially, a combination of cyclophosphamide, vincristine, and prednisone with or without doxorubicin is a reasonable choice. The use of fludarabine has been reported in two patients with  $\mu$ -HCD; one had an "apparent hematologic response,"<sup>64</sup> and the other had a partial response.<sup>56</sup> Vincristine, cyclophosphamide, prednisolone, and doxorubicin in combination with rituximab led to complete resolution of tumoral lesions in one patient with  $\mu$ -HCD.<sup>65</sup>

## COURSE AND PROGNOSIS

The course of  $\mu$ -HCD is variable. Because of the rarity of the disease, no large series of patients treated systematically in a single center has been reported. The median duration of survival from the time of diagnosis is 24 months (range: <1 month to 11 years).<sup>54</sup> Because several of the reported patients had findings consistent with  $\mu$ -HCD before recognition of the  $\mu$ -HCD protein, the course is probably longer than reported in most patients. In one patient, the hematologic abnormalities became normal and the  $\mu$ -HC disappeared after 2 years without specific treatment.



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